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Interleukin-12 Receptor β 1 or Interleukin-12 Receptor β 2 deficiency in mice indicates that Interleukin-12 and Interleukin-23 are not essential for host recovery from viral encephalitis.¹

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Abstract

Vesicular Stomatitis Virus (VSV), a negative sense, single-stranded RNA Rhabdovirus causes acute viral encephalitis when administered intranasally to mice. Interleukin-12 (IL-12) is a key pro-inflammatory cytokine, produced largely by the antigen presenting cells (APC) that bridges the innate and acquired immune responses. IL-12 is efficacious in enhancing recovery from VSV infection of the murine CNS. This effect is mediated by nitric oxide (NO) produced by the neuronal isoform of nitric oxide synthase (NOS-1), and is independent of the proinflammatory cytokines IFN- γ and TNF- α . These data implied a link between IL-12 and NOS-1.

Here we investigate the role of the IL-12R during VSV pathogenesis, using IL-12R β 2 and IL-12R β 1-deficient mice. We showed that a deficiency in either IL-12R β 2 or IL-12R β 1 had no effect on the outcome of VSV infection of the CNS or on the clearance of VSV from the CNS. Further these data indicate that IL-23 is not acting redundantly in the absence of IL-12 during VSV-induced encephalitis.

Keywords

VSV; IL-12R; IL-12; CNS; inflammation

IL-12 is a pleiotropic cytokine at the cusp between the innate and acquired immune response. This pro-inflammatory cytokine is secreted by antigen-presenting cells and phagocytes, including dendritic cells, macrophages and neutrophils (1,5,16). In the CNS, astrocytes and microglia are the principle sources of IL-12 (4,19).

The IL-12R is a heterodimer consisting of subunits designated IL-12R β 1 and IL-12R β 2 (3, 18). Cells of hematopoietic origin express the IL-12R, including NK cells, activated T-cells and dendritic cells (7,18,26). Microglia, have been demonstrated to express the IL-12R, *in vitro* (20) (21). Our lab has recently demonstrated the expression of a functional IL-12R by CNS-derived neurons, *in vitro* (9).

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In acquired immunity, the primary role of IL-12 is to drive the differentiation of CD4⁺ T-helper (T_H) cells towards a T_H1 phenotype (24). Naïve T-cells express the IL-12Rβ1 subunit at constitutive levels, where the expression of the IL-12Rβ2 subunit is tightly regulated in response to the local cytokine environment. The expression of the IL-12Rβ2 on the surface of T_H0 cells, initiated by IL-2, IL-12 and IFN-γ (23) is required for IL-12 responsiveness, and therefore the differentiation of these cells to a T_H1 phenotype (6,10). These cells are critical in immune responses to intracellular pathogens.

In innate immunity, IL-12 principally functions through NK cells, recruiting them to a region of active infection, stimulating them; enhancing their cytotoxic activity and IFN-γ production (22). NK cells constitutively express both IL-12Rβ1 and IL-12Rβ2 subunits, presumably to allow a rapid response to secreted IL-12 without *de novo* synthesis of IL-12R subunits (23). IFN-γ produced by NK cells in response to IL-12 upregulates the production of IL-12 by APC and phagocytes in a positive feedback loop (13-15,28).

Vesicular Stomatitis Virus (VSV) is an enveloped negative sense single stranded RNA virus. Intranasal administration of VSV to mice results in an acute, fatal viral encephalitis. We have shown that treatment of mice with exogenous IL-12 results in enhanced survival from VSV infection (11), in an IFN-γ independent mechanism (11). The beneficial effects of IL-12 treatment are only observed when IL-12 is given within the first 48 hours post-infection, indicating that a rapid, early innate response is required for IL-12-mediated inhibition of VSV replication in the CNS (8,11). The efficacy of IL-12 treatment is mediated by nitric oxide (NO), released by nitric oxide synthase-1 (NOS-1), the neuron specific isoform of NOS (12). A deficiency in NOS-1 abrogated the beneficial effects of exogenous IL-12 during VSV infection (12). These data indicated a possible link between IL-12 treatment and NOS-1. Recent data from our lab has also demonstrated that expression of a functional IL-12R is sufficient and required for IL-12 mediated anti-viral responses by neurons, *in vitro* (9). Therefore, we hypothesized that a deficiency in IL-12 responsiveness due to the loss of a functional IL-12R would result in the exacerbation of VSV pathology as was observed in NOS-1 deficient animals.

We tested this hypothesis and the role of the IL-12R in VSV pathogenesis using IL-12Rβ2-deficient C57BL/6J mice obtained from Jackson Labs (Bar Harbor, ME). These mice are known to have the same number of circulating naïve T-cells as congenic wild-type mice, however the number of activated T-cells responding to an immune challenge is significantly diminished (26). Furthermore, reduced NK cell activity, serum IFN-γ concentration and a lack of STAT-4 phosphorylation, indicated that these mice are unable to respond to IL-12 (26).

We intranasally infected 7 week old IL-12Rβ2-deficient mice and age/sex matched congenic controls (Charles River Labs, MA) with 1.0×10^2 pfu VSV as previously described (11). The animals were monitored every other day during the course of infection, observing mortality and morbidity (change in mass) in four independent experiments (Figure 1). The results of these studies indicate that IL-12Rβ2-deficient mice do not show a significant increase in morbidity or mortality (Figure 1); demonstrating that a deficiency in IL-12 responsiveness does not alter the outcome of VSV-induced encephalitis. Therefore, although exogenous IL-12 treatment results in the inhibition viral replication and enhanced recovery from VSV infection, endogenous IL-12 responses are not absolutely required to protect mice from VSV pathogenesis.

To confirm these animals are unresponsive to IL-12 during VSV pathogenesis, we tested the response of IL-12Rβ2-deficient animals to exogenous mrIL-12 treatment. Given the lack of IL-12 responsiveness described in these mice (27), no benefit from IL-12 treatment during VSV infection was expected. VSV infected, IL-12Rβ2-deficient and congenic age/sex matched C57BL/6J control mice intranasally with VSV. At the time of infection, half of the mice were

treated with an intraperitoneal injection of 200 ng mrIL-12 (Atlanta Biologicals, GA) while the other half received injections of the diluent alone. At the indicated times p.i. (Figure 2), the mice were sacrificed and whole brain homogenates were collected. The titer of VSV in these homogenates was determined by plaque assay, as previously described (11).

Comparing the viral titer in the CNS of IL-12 treated wild-type mice to those that received diluent alone, there was a modest, but significant decrease in VSV titer (Figure 2), indicating that these mice responded to exogenous IL-12 treatment, as expected. Conversely, mrIL-12 treated, IL-12R β 2-deficient mice did not demonstrate the same inhibition of VSV replication in the CNS. These data show that IL-12R β 2-deficient animals do not respond to exogenous mrIL-12 treatment during VSV pathogenesis, as expected. However, the loss of a functional IL-12R β 2 and therefore endogenous IL-12 responses had no effect on the outcome of VSV infection. These data imply that there is/are redundant pathway(s) compensating for the loss of IL-12 responsiveness in these mice.

Recently discovered members of the new heterodimeric cytokine family are attractive candidates as redundant molecules in the absence of IL-12 function, given their similarities. For example, IL-23, (17) has genetic, structural, signal transduction and functional similarities to IL-12 in the acquired immune response. Each of these heterodimeric cytokines pairs a different smaller subunit with the IL-12 p40 subunit (17). Furthermore, they also share the IL-12R β 1 subunit in their heterodimeric receptors, and both receptors subsequently signal through the JAK/STAT transduction pathways (17). These similarities may allow IL-23 to drive the transcription of genes also regulated by IL-12.

Given that the IL-12R β 1 is sufficient and required for high affinity binding and function of both IL-12 and IL-23, a deficiency in the IL-12R β 1 subunit will result in the loss of both IL-12 and IL-23 responsiveness. IL-12R β 1-deficient mice (25) are known to have reduced NK and T-cell responses against endotoxin exposure *in vivo*, and have a phenotype similar to what has been described above for IL-12R β 2-deficient animals. The phenotype of IL-12R β 1-deficient mice is also similar to the phenotype observed in IL-12 p40 deficient mice (25).

We hypothesized that if IL-23 were acting in the absence of IL-12 responsiveness in IL-12R β 2-deficient mice, then an IL-12R β 1 deficiency would result in exacerbated VSV-induced encephalitis. This hypothesis was supported by studies that showed IL-12R β 1-deficient mice are susceptible to EAE where IL-12R β 2-deficient mice of the same strain were resistant (29,30), indicating that IL-23 was acting in the absence of IL-12 responsiveness to activate encephalogenic T-cells in the EAE model.

We compared the susceptibility of IL-12R β 1-deficient mice to intranasal VSV infection to that of congenic age/sex matched C57BL/6J mice (Charles River,), as described above for IL-12R β 2-deficient animals, in four independent studies. The IL-12R β 1-deficient animals showed no increased susceptibility to VSV infection (Figure 3). These data indicate that the loss of both IL-12 and IL-23 responsiveness has no effect on the outcome of VSV infection. Therefore, IL-23 is not acting redundantly in the absence of IL-12 responsiveness in a VSV-induced encephalitis model.

As described above, IL-12R β 1 and congenic age/sex matched controls were tested for responses to exogenous IL-12 treatment. The titer of infectious VSV in whole brain homogenates of mrIL-12-treated and diluent-treated mice was determined by plaque assay at the indicated time points p.i. (Figure 4). As expected, the IL-12-treated wild-type mice showed a modest but statistically significant inhibition of VSV replication, when compared to the diluent treated controls (Figure 4). The IL-12R β 1-deficient animals did not show the same inhibition with IL-12 treatment, when compared to diluent treated controls. These data confirm that a deficiency in endogenous IL-12 responses has no effect on the outcome of VSV

pathogenesis; and further that IL-23 is not acting redundantly in the absence of a functional IL-12 response.

This study was designed to test whether the functional IL-12R *in vivo* is sufficient and required for the inhibition of VSV replication and a concomitant decrease in the severity of VSV-induced encephalitis in the CNS of mice. Previous work from our lab has shown that treatment with exogenous IL-12 treatment results in enhanced recovery from VSV infection in the CNS and that this benefit is mediated by NO produced by NOS-1 (12).

The experiments described above clearly indicate that although IL-12 has a beneficial effect when delivered exogenously (8,11,12), the lack of endogenous IL-12 responsiveness due to a deficiency in a functional IL-12R has no effect on the outcome of VSV pathogenesis. Supporting these findings, previous work published by our lab has shown that a deficiency in STAT4, the principle signal transduction molecule in the IL-12 pathway, does not result in increased susceptibility to VSV infection (2). Data presented in this study eliminates the possibility that IL-12 was signalling through an alternative pathway, such as NF- κ B, which has been associated with IL-12 responses in dendritic cells (7).

We also observed no change in inflammatory cytokine or chemokine expression, using ribonuclease protection assays; or in the relative number of CD4 or CD8-positive T-cells infiltrating into the CNS parenchyma when comparing IL-12R deficient and wild-type control animals (unpublished data), indicating that there is no significant change to the inflammatory response to VSV infection with the loss of IL-12 responsiveness. No change in the outcome of VSV pathogenesis or in the inflammatory response to the virus with IL-12 deficiency would indicate that redundant mechanisms act in the absence of IL-12 to control VSV replication.

Redundant mechanisms are a common theme in immunology and are extremely important to protect the host from infections when a portion of the immune response fails, or is circumvented by an adaptation of the pathogen. Cytokines with closely related signal transduction pathways make interesting candidates for IL-12 redundant cytokines, as the activated transcription factors may target the same genes. IL-23 and IL-27 are both members of the same heterodimeric cytokine family as IL-12 and share many characteristics. Data presented in this study using IL-12R β 1-deficient animals (Figures 3, 4) eliminate IL-23 as a candidate. This study does not eliminate the possibility that intraperitoneal treatment with IL-23, like IL-12, may be beneficial though not required for recovery from VSV-induced encephalitis. At the time of writing, neither the reagents necessary to test the efficacy of exogenous IL-23 treatment; nor the reagents and mice necessary to test IL-27 redundancy were not available at the time these studies were conducted.

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Figure 1.

IL-12R β 2-deficient animals are not more susceptible to VSV-induced encephalitis. IL-12R β 2-deficient (IL-12R β 2^{-/-}) and C57BL/6J control mice were infected intranasally with VSV and monitored every other day for morbidity and mortality during the course of VSV pathogenesis. Mortality was monitored daily until no pathology was observed in the surviving animals. These data are the combination of 4 identical, independent experiments.

Figure 2.

IL-12R β 2 deficient mice are not responsive to exogenous IL-12. C57BL/6J (WT) and IL-12R β 2 deficient (12R β 2) mice were infected intranasally with VSV. At day 3 pi, the animals were euthanized and their CNS collected and homogenized. The amount of virus in the CNS homogenates was determined by plaque assay. These data are presented as the geometric mean ($n=6$) $\pm$ geometric error and represent 2 independent experiments. Treatment groups compared using a two-tailed t-test (* = $p < 0.05$).

Figure 3.

IL-12Rβ1-deficient animals are not more susceptible to VSV-induced encephalitis.

IL-12Rβ1-deficient (IL-12Rβ1^{-/-}) and C57BL/6J control mice were infected intranasally with VSV and monitored every other day for (A) morbidity and (B) mortality during the course of VSV pathogenesis. (A) Morbidity was monitored by the weight of the animal. The indicated weight is the average weight of all animals monitored (n = 22); error bars represent standard error. Monitoring was continued until the animals showed full recovery from the infection, indicated by a return to pre-infection weights. These data are representative of 4 independent experiments. (B) Mortality was monitored daily until no pathology was observed in the surviving animals. These data are the combination of 4 identical, independent experiments.

Figure 4.

IL-12Rβ1-deficient mice are not responsive to exogenous IL-12. C57BL/6J (WT) and IL-12Rβ1 deficient (12Rβ1) mice were infected intranasally with VSV. At day 3 pi, the animals were euthanized and their CNS collected and homogenized. The amount of virus in the CNS homogenates was determined by plaque assay. These data are presented as the geometric mean ($n=6$) $\pm$ geometric error and represent 2 individual experiments. Treatment groups compared using a two-tailed t-test (* = $p < 0.05$).