Interleukin-12 (IL-12) and IL-18 Are Important in Innate Defense against Genital Herpes Simplex Virus Type 2 Infection in Mice but Are Not Required for the Development of Acquired Gamma Interferon-Mediated Protective Immunity

ALI M. HARANDI,1 BO SVENNERHOLM,2 JAN HOLMGREN,1 AND KRISTINA ERIKSSON1*
Departments of Medical Microbiology and Immunology1 and Clinical Virology,2 Göteborg University, 413 46 Göteborg, Sweden

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Using a combination of gene-targeted mice and neutralizing antibodies, we showed that interleukin-12 (IL-12) and IL-18 are important in the innate control of genital herpes simplex virus type 2 infection but were not found to be critical, either singly or in combination, for the development of a protective gamma interferon-mediated immune response.

Natural antibodies, NK cells, neutrophils, macrophages, and complement all contribute to the innate control of genital herpes infection (1, 5, 9, 13, 24). Once a herpes simplex virus type 2 (HSV-2) infection is established, virus-specific CD4+ and CD8+ T cells develop and participate in the resolution of the infection (16). To prevent infection, both specific antibodies and T cells are implicated. Antibodies limit the uptake and replication of the virus (30). Thereafter, memory T cells infiltrate the exposed area (26, 32).

Gamma interferon (IFN-γ) plays an important role in T-cell-mediated viral clearance (25, 33). There is a markedly increased genital virus load in vaccinated mice treated with anti-IFN-γ antibodies (25, 33). Furthermore, lack of protection in vaccinated CD4−/− mice correlates with reduced IFN-γ responses, and protection can be restored in vivo by addition of exogenous IFN-γ (13).

Interleukin-12 (IL-12) and IL-18 are key factors for Th1 development. IL-12 is the dominant factor inducing IFN-γ production by T cells and NK cells (27). IL-18 synergizes with IL-12 in inducing IFN-γ by T cells and is thus required for optimal IFN-γ synthesis (18, 34, 38, 39). Previous studies in experimental animals point to the important role of IL-12 and IL-18 in host defense against intracellular bacteria, parasites, and fungi (6, 11, 12, 15, 20–22, 28). To assess the requirements of IFN-γ, IL-12, and IL-18 in innate immune control of genital HSV-2 infection, C57BL/6 wild-type (WT), IFN-γ−/− (10), IL-12p40−/− (19), and IL-18−/− (40) mice were vaccinated with an attenuated strain of HSV-2 strain 333 (37). Following HSV-2 infection, vaginal fluids were collected and HSV-2 titers were determined by plaque assay, and mice were examined daily for disease and death. Statistical analyses were done by Student’s t test or log rank test.

Innate defense against primary infection. Three days after viral inoculation the level of shed virus was four times higher in IFN-γ−/− mice (Fig. 1A) and these animals died significantly earlier (4 days) than WT mice (P < 0.01) (Fig. 1B). The vaginal HSV-2 titers both in IL-12−/− and in IL-18−/− mice were higher than those observed for WT animals (Fig. 1A), and the animals died significantly earlier (3 days) [P < 0.05] in IL-12−/− mice and 4 days [P < 0.01] in IL-18−/− mice (Fig. 1B).

The most prominent function of IL-12 and IL-18 in innate defense is as enhancers of NK cell activity including IFN-γ production (42, 43). IL-18 can also induce intercellular adhesion molecule 1 expression by an IFN-γ-independent pathway, promoting immune-cell recruitment to the target tissue (17). To assess the outcome of primary genital HSV-2 infection in the absence of both IL-12 and IL-18, we depleted endogenous IL-18 in IL-12−/− mice. A neutralizing rat anti-mouse IL-18 antibody (R&D systems) (20 μg/mouse) was administered intraperitoneally to IL-12−/− mice 4 h prior to vaginal HSV-2 inoculation. An additional 10 μg of anti-IL-18 antibody was given vaginally at the time of inoculation followed by 20 μg of anti-IL-18 antibody given intraperitoneally on days 2, 4, and 6 after virus challenge. The vaginal HSV-2 titers in anti-IL-18 antibody-treated IL-12−/− mice were threefold higher than those in control antibody (rat immunoglobulin G [IgG])-treated IL-12−/− mice on day 3 postchallenge (Fig. 1C), and these mice also died significantly earlier (3 days) (P < 0.05) (Fig. 1D).

The natural defense against genital HSV-2 infection is impaired in mice lacking IL-12 and/or IL-18.

Vaccination-induced acquired defense. Vaginal vaccination of mice with an attenuated strain of HSV-2 confers protection against a lethal challenge with a virulent strain of the virus (23, 31). To examine the roles of IFN-γ, IL-12, and IL-18 for the development of vaccine-induced protective immune responses, IFN-γ−/−, IL-12−/−, and IL-18−/− mice were vaccinated with 3.6 × 106 PFU of attenuated HSV-2 strain Lyon, which contains a partial deletion of the thymidine kinase gene (2), and then 4 weeks later they were challenged vaginally with a lethal dose of HSV-2. Three days after the challenge infection, no viral replication was detected in vaccinated WT mice and consequently no death was observed (Fig. 1E). In contrast, vaccinated IFN-γ-deficient mice had evidence of persistent viral...
FIG. 1. Vaginal HSV-2 titers and disease progression in mice deficient in IFN-γ, IL-12, or IL-18 after primary and secondary genital HSV-2 infections. (A and B) Naïve mice were challenged intravaginally with a lethal dose of HSV-2, and the vaginal HSV-2 titers (A) were examined on day 3 after viral challenge (n = 6). Differences were statistically significant at P values of <0.05 (*) and <0.01 (**) by Student’s t test compared with WT mice. The mice were monitored daily for mortality (n ≥ 12) (B). (C and D) Effects of in vivo administration of neutralizing anti-IL-18 antibody on vaginal HSV-2 replication and disease progression in HSV-2-challenged IL-12−/− mice. Groups of IL-12−/− mice (4 mice/group) received either neutralizing anti-IL-18 antibody or purified normal IgG2a on days 0, 2, 4, and 6 after HSV-2 challenge. At day 3 after viral challenge, the vaginal HSV-2 titers (C) were evaluated. *, statistically significant at P values of <0.05 compared to control antibody-treated IL-12−/− mice. The mice were examined daily for mortality (D). (E) Survival of vaccinated C57BL/6 WT, IFN-γ−/−, IL-12−/−, IL-18−/− (10 to 15 mice/group), and IL-18-depleted IL-12−/− mice (6 mice/group) after a lethal challenge with HSV-2.
replication (134 ± 47.9 [mean ± standard error of the mean] PFU) and the majority of the vaccinated IFN-γ−/− mice had died by day 20 (Fig. 1E). No viral replication was observed in the vaccinated IL-12−/− or IL-18−/− mice on day 3 postchallenge, and all these animals survived (Fig. 1E).

Next, we examined the induction of protective immunity in the absence of both IL-12 and IL-18. Endogenous IL-18 was depleted in IL-12−/− mice by using different sets of anti-IL-18 antibodies (rat and goat) at the time of vaccination and at the time of challenge as described above. Similarly to what

FIG. 2. HSV-2-specific immune responses in vaccinated WT, IFN-γ−/−, IL-12−/−, and IL-18−/− mice (n = 4 to 8). (A to C) Spleen mononuclear cells obtained 4 weeks postvaccination were cultured in the presence of either UV-inactivated HSV-2 or mock antigen and analyzed for HSV-2-specific production of IFN-γ and IL-2. Data are expressed as a stimulation index (A), the concentration (in picograms per milliliter) of secreted IL-2 (B), and the concentration (in picograms per milliliter) of secreted IFN-γ (C) per million analyzed spleen cells. ND, not detected. (D) HSV-2-specific DTH reactions were measured 4 weeks after vaccination. Results are expressed as the mean and standard error of the mean of the HSV-2-specific DTH reaction (Δ mm, 102) at 48 h postchallenge. (E) Ratio of HSV-specific IgG2a to IgG1 in WT, IFN-γ−/−, IL-12−/−, and IL-18−/− mice 4 weeks after vaccination with attenuated HSV-2. Data are expressed as the mean and standard error of the mean. Differences were statistically significant at P values of <0.05 (*) and <0.01 (**) by Student’s t test compared with vaccinated WT mice.
was observed for vaccinated WT mice, no viral replication was observed on day 3 postchallenge in anti-IL-18-treated IL-12−/− mice. Neither the vaccinated anti-IL-18-treated IL-12−/− mice nor the control antibody-treated IL-12−/− animals died or exhibited any signs of disease throughout the 20-day observation course (Fig. 1E). These results demonstrate that IFN-γ but not IL-12 or IL-18 is required for development of acquired protective immunity against genital HSV-2 infection.

**Immune factors associated with protection.** The immunity levels of mice deficient in IFN-γ, IL-12, or IL-18 were compared 4 weeks postvaccination. The production of type 1 cytokines (IFN-γ and IL-2) in vitro was examined using a cellELISA method (13). Spleen cells from all groups of vaccinated mice responded to in vitro recall HSV-2 antigen with a strong proliferative response (Fig. 2A) and IL-2 production (Fig. 2B), even though the responses were lower in IL-12−/− and IL-18−/− mice. There were significantly reduced levels of IFN-γ in spleen cells from vaccinated IL-12−/− mice (<0.01), whereas the levels of IFN-γ in spleen cells from IL-18−/− mice were comparable to those of WT animals (Fig. 2C). Thus, an appreciable Th1 type response developed in IL-18−/− animals after vaccination whereas IL-12−/− mice displayed an impaired Th1 type response.

We also examined the HSV-2-specific delayed-type hypersensitivity (DTH) 4 weeks after vaccination. The specific footpad swelling was examined 48 h after injection of UV-inactivated HSV-2 (corresponding to 7 × 10⁶ PFU) or mock antigens in the left and right footpads, respectively. In IL-18−/− mice, the DTH response was of a magnitude similar to that in vaccinated WT mice (Fig. 2D). The IL-12−/− mice had intermediate levels of DTH response, whereas IFN-γ−/− mice showed an almost completely abolished DTH response (Fig. 2D). Thus, protection in the vaccinated animals was associated with a maintained capacity to mount HSV-2-specific IFN-γ responses in vitro and DTH responses in vivo. Our results support and extend previous findings that IFN-γ production is important in protective immunity against genital HSV-2 infection (13, 25, 33). However, it was evident that an optimal Th1 response required IL-12. These findings are in line with other observations implying that IFN-γ production and a Th1-type immune response can be induced during certain viral infections even in the absence of IL-12 (29, 36, 44). Other factors can compensate for the lack of IL-12. IL-18 cannot induce Th1 development by itself (34) but can contribute to IFN-γ response through activation of the IFN-γ promoter in T cells (4). The strong Th1 immune response in IL-18−/− mice was likely induced by IL-12 in synergy with other cytokines such as IL-15, tumor necrosis factor alpha, and IL-1β (3, 7, 8, 41). HSV-specific serum IgG was measured in sera obtained 4 weeks postvaccination using an enzyme-linked immunosorbent assay based on a deoxycholate-solubilized membrane fraction of HSV-1-infected cells (14). The serum levels of HSV-specific IgG antibodies were comparable in all groups of vaccinated mice (not shown), but the ratio of HSV-specific IgG2a to IgG1 varied considerably. WT and IL-12−/− mice had high levels of HSV-specific IgG2a resulting in a significant IgG2a/IgG1 ratio. IFN-γ−/− and IL-18−/− mice, on the other hand, had impaired HSV-specific IgG2a levels and thus gave a diminished IgG2a/IgG1 ratio (Fig. 2E). To our knowledge, the role of IL-18 as an important switch factor for antigen-specific IgG2a subclasses in vivo has not been demonstrated previously. This finding correlates with the documented role of NK cells in the development of an IgG2a response (35), as IL-18 is an important activator of NK cells (40).

In conclusion, our results show that IFN-γ plays a key role in both innate and acquired immunity to genital HSV-2 infection, while IL-12 and IL-18 are important for innate but not for vaccination-induced adaptive immunity. The latter finding raises interesting questions about the nature of factors other than IL-12 and IL-18 that are induced by viral infection and contribute to the development of protective IFN-γ production in the adaptive immune response.

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