JOURNAL OF VIROLOGY, July 2001, p. 6705–6709 Vol. 75, No. 14
0022-538X/01/$04.00+0 DOI: 10.1128/JVI.75.14.6705–6709.2001

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 ERIKSSON[1]*

Departments of Medical Microbiology and Immunology[1] and Clinical Virology,[2] Göteborg University, 413 46 Göteborg, Sweden

Received 8 January 2001/Accepted 25 April 2001

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) appears to play 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-$\gamma^{-/-}$ (10), IL-12p40$^{-/-}$ (19), and IL-18$^{-/-}$ (40) mice were vaginally challenged with a lethal dose (4×10^4 PFU) 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-$\gamma^{-/-}$ 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). Thus, 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-$\gamma^{-/-}$, IL-$12^{-/-}$, and IL-$18^{-/-}$ mice were vaccinated with 3.6×10^6 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

* Corresponding author. Mailing address: Department of Medical Microbiology & Immunology, Guldhedsgatan 10A, 413 46 Göteborg, Sweden. Phone: (46) 31-3424761. Fax: (46) 31-820160. E-mail: kristina.eriksson@microbio.gu.se.

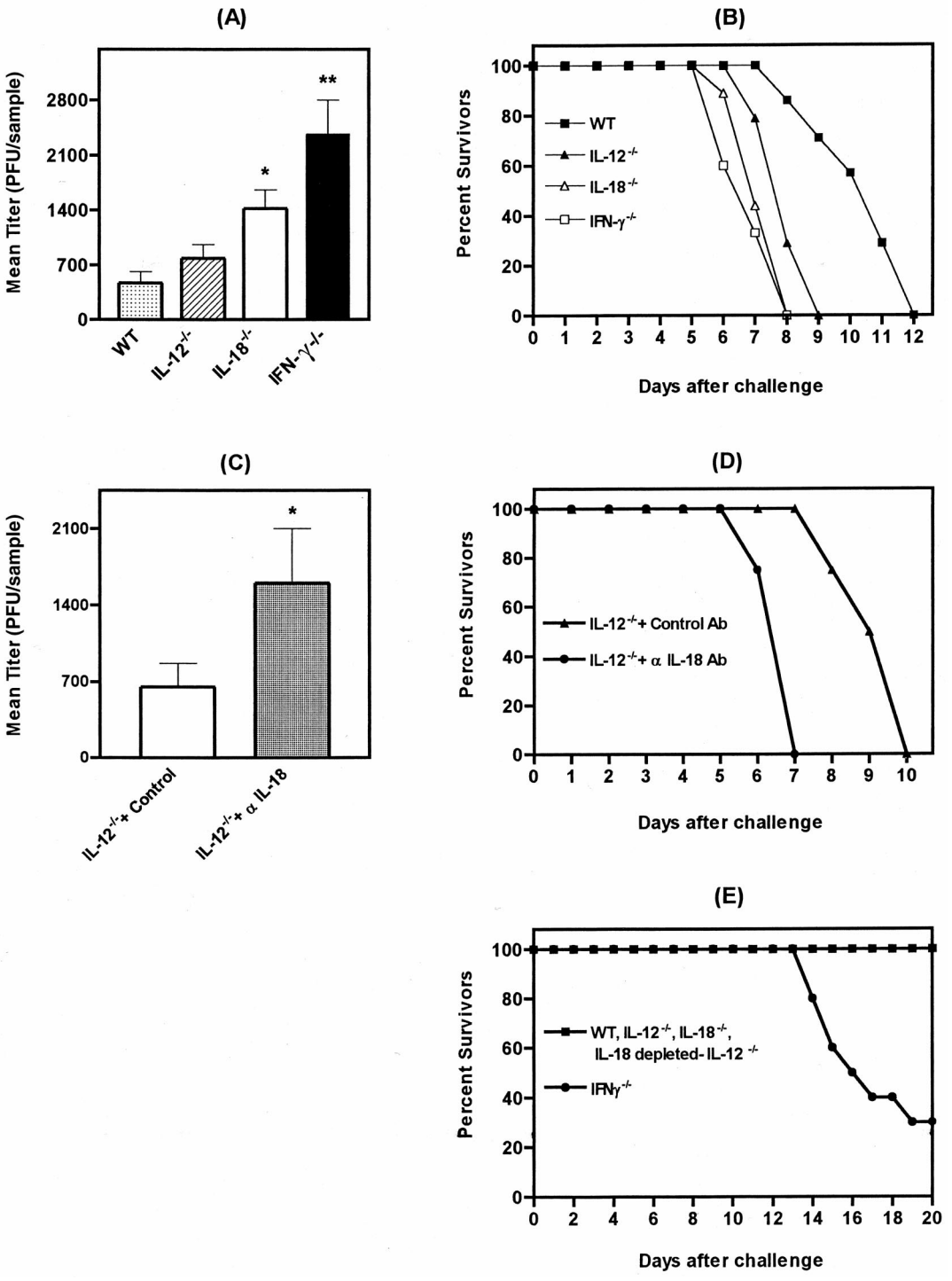

FIG. 1. Vaginal HSV-2 titers and disease progression in mice deficient in IFN-γ, IL-12, or IL-18 after primary and secondary genital HSV-2 infections. (A and B) Naïve mice were challenged intravaginally with a lethal dose of HSV-2, and the vaginal HSV-2 titers (A) were examined on day 3 after viral challenge ($n = 6$). 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 ($n \geq 12$) (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.

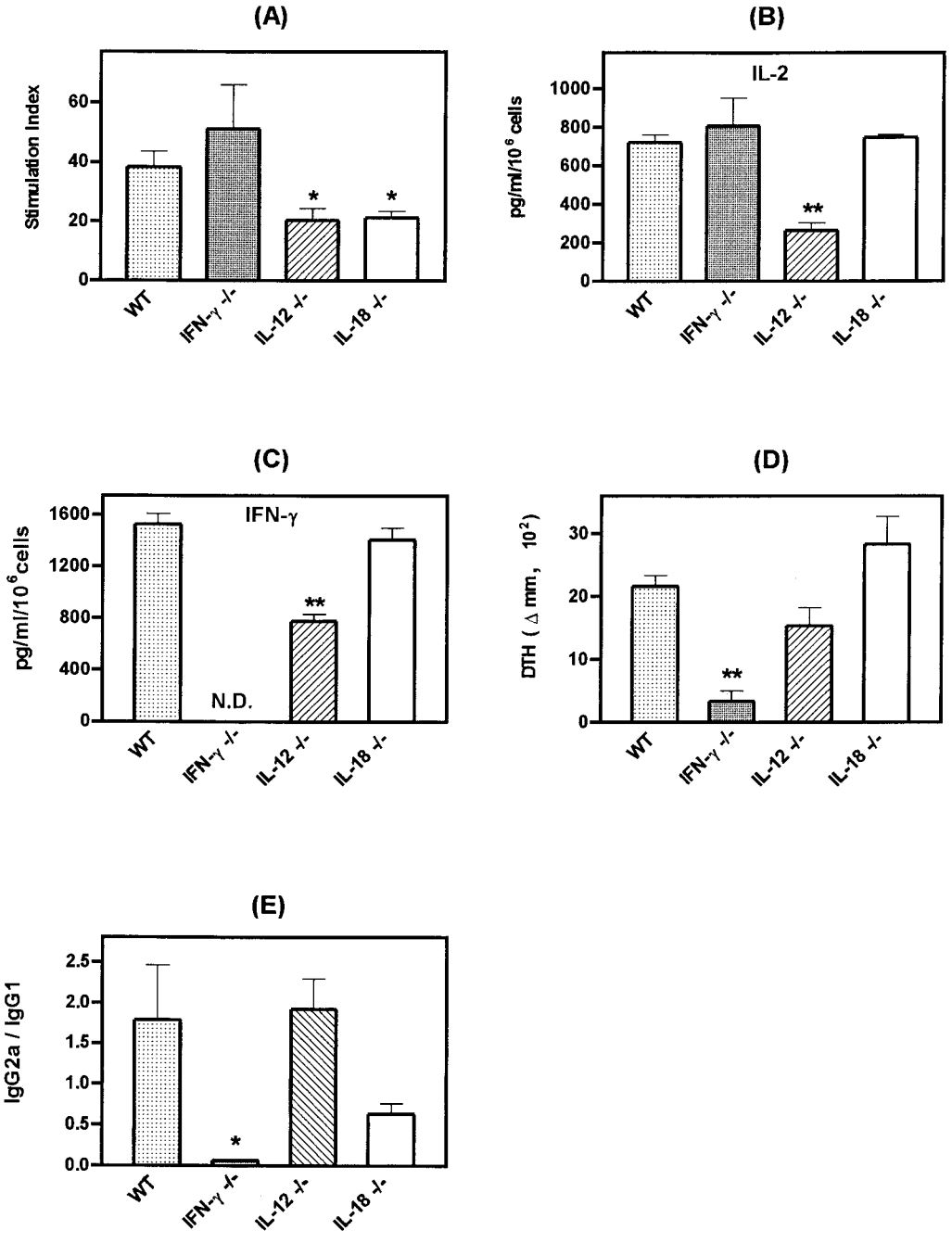

FIG. 2. HSV-2-specific immune responses in vaccinated WT, IFN-$\gamma^{-/-}$, IL-$12^{-/-}$, and IL-$18^{-/-}$ mice (n = 4 to 8). (A to C) Spleen mononuclear cells obtained 4 weeks postvaccination were cultured in the presence of either UV-inactivated HSV-2 or mock antigen and analyzed for HSV-2-specific 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, 10^2) at 48 h postchallenge. (E) Ratio of HSV-specific IgG2a to IgG1 in WT, IFN-$\gamma^{-/-}$, 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.

replication (134 ± 47.9 [mean ± standard error of the mean] PFU) and the majority of the vaccinated IFN-$\gamma^{-/-}$ 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

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 ($P < 0.01$), 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 7×10^6 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.

We are grateful to Inger Nordström for skilled technical assistance. We also thank Pia Axelsson for breeding the gene-targeted mice. We gratefully acknowledge Shizuo Akira and Kiyoshi Takeda, Hyogo College of Medicine, Japan, for providing the IL-18$^{-/-}$ mice.

This study was supported by the Swedish Medical Research Council, SIDA/SAREC's Special Program for AIDS and Related Diseases, The Swedish Strategic Foundation Program in Infection and Vaccinology, Socialstyrelsens Fonder, The Swedish Society for Medical Research, Wilhelm and Martina Lundgrens Science Foundation, Magn Bergvalls' Foundation, and the LUA Foundation at Sahlgren's Hospital.

REFERENCES

1. **Adler, H., J. L. Beland, N. C. Del-Pan, L. Kobzik, R. A. Sobel, and I. J. Rimm.** 1999. In the absence of T-cells, natural killer cells protect from mortality due to HSV-1 encephalitis. J. Neuroimmunol. **93:**208–213.
2. **Andrei, G., R. Snoeck, and E. De Clerq.** 1997. Differential susceptibility of several drug-resistant strains of herpes simplex virus type 2 to various antiviral compounds. Antivir. Chem. Chemother. **8:**457–461.
3. **Avice, M.-N., C. Demeure, G. Delespesse, M. Rubio, M. Armant, and M. Sariati.** 1998. IL-15 promotes IL-12 production by human monocytes via T cell-dependent contact and may contribute to IL-12-mediated IFN-gamma secretion by CD4$^+$ T cells in the absence of TCR ligation. J. Immunol. **161:**3408–3415.
4. **Barbulescu, K., C. Becker, J. Schlaak, E. Schmitt, K.-H. Buschenfelde, and M. Neurath.** 1998. Cutting edge: IL-12 and IL-18 differentially regulate the transcriptional activity of the human IFN-gamma promoter in primary CD4$^+$ T lymphocytes. J. Immunol. **160:**3642–3647.
5. **Benencia, F., and M. C. Courreges.** 1999. Nitric oxide and macrophage antiviral extrinsic activity. Immunology **98:**363–370.
6. **Cai, G., R. Kastelein, and C. Hunter.** 2000. Interleukin-18 (IL-18) enhances innate IL-12-mediated resistance to *Toxoplasma gondii*. Infect. Immun. **68:** 6932–6938.
7. **Cai, G., R. A. Kastelein, and C. A. Hunter.** 1999. IL-10 enhances NK cell proliferation, cytotoxicity and production of IFN-gamma when combined with IL-18. Eur. J. Immunol. **29:**2658–2665.
8. **Carson, W., M. Ross, R. Baiocchi, M. Marien, N. Boiani, K. Grabstein, and M. Caligiuri.** 1995. Endogenous production of interleukin 15 by activated human monocytes is critical for optimal production of interferon-gamma by natural killer cells in vitro. J. Clin. Investig. **96:**2578–2582.
9. **Da Costa, X. J., M. A. Brockman, E. Alicot, M. Ma, M. B. Fischer, X. Zhou, D. M. Knipe, and M. C. Carroll.** 1999. Humoral response to herpes simplex virus is complement dependent. Proc. Natl. Acad. Sci. USA **96:**12708–12712.
10. **Dalton, D., S. Pitt-Meek, I. Keshav, A. Figari, Bradley, and T. Stewart.** 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science **259:**1739.
11. **Decken, K., G. Kohler, K. Palmer-Lehmann, A. Wunderlin, F. Mattner, J. Magram, M. Gately, and G. Alber.** 1998. Interleukin-12 is essential for a protective Th1 response in mice infected with *Cryptococcus neoformans*. Infect. Immun. **66:**4994–5000.
12. **Gazzinelli, R., M. Wysocka, S. Hyashi, E. Denkers, S. Hieny, P. Caspar, G. Trinchieri, and A. Sher.** 1994. Parasite-induced IL-12 stimulates early IFN-gamma synthesis and resistance during acute infection with Toxoplasma gondii. J. Immunol. **153:**2533–2543.
13. **Harandi, A. M., B. Svennerholm, J. Holmgren, and K. Eriksson.** 2001. Differential roles of B-cells and IFN-γ-secreting CD4$^+$ T-cells in innate and adaptive immune control of genital HSV-2 infection in mice. J. Gen. Virol. **82:**845–853.
14. **Jeansson, S., M. Forsgren, and B. Svennerholm.** 1983. Evaluation of solubilized herpes simplex virus membrane antigen by enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18:**1160–1166.
15. **Kawakami, K., Y. Koguchi, M. Qureshi, Y. Kinjo, S. Yara, A. Miyazato, M. Kurimoto, K. Takeda, S. Akira, and A. Saito.** 2000. Reduced host resistance

and Th1 response to Cryptococcus neoformans in interleukin-18 deficient mice. FEMS Microbiol. Lett. **186:**121–126.
16. **Koelle, D. M., C. M. Posavad, G. R. Barnum, M. L. Johnson, J. M. Frank, and L. Corey.** 1998. Clearance of HSV-2 from recurrent genital lesions correlates with infiltration of HSV-specific cytotoxic T lymphocytes. J. Clin. Investig. **101:**1500–1508.
17. **Kohka, H., T. Yoshini, H. Iwagaki, I. Sakuma, T. Tanimoto, Y. Matsuo, M. Kurimoto, K. Orita, T. Akagi, and N. Tanaka.** 1998. Interleukin-18/interferon-gamma-inducing factor, a novel cytokine, up-regulates ICAM-1 (CD54) expression in KG-1 cells. J. Leukoc. Biol. **64:**519–527.
18. **Kohno, K., J. Kataoka, T. Ohtsuki, Y. Suemoto, I. Okomoto, M. Usui, M. Ikeda, and M. Kurimoto.** 1997. IFN-γ-inducing factor (IGIF) is a costimulatory factor on the activation of Th1 but not Th2 cells and exerts its effect independently of IL-12. J. Immunol. **158:**1541–1550.
19. **Magram, J., S. E. Connaughton, R. R. Warrier, D. M. Carvajal, C. Y. Wu, J. Ferrante, C. Stewart, U. Sarmiento, D. A. Faherty, and M. K. Gately.** 1996. IL-12-deficient mice are defective in IFN gamma production and type 1 cytokine responses. Immunity **4:**471–481.
20. **Mastroeni, P., S. Clare, S. Khan, J. Harrison, C. Hormaeche, H. Okamura, M. Kurimoto, and G. Dougan.** 1999. Interleukin 18 contributes to host resistance and gamma interferon production in mice infected with virulent *Salmonella typhimurium*. Infect. Immun. **67:**478–483.
21. **Mastroeni, P., J. Harrison, J. Chabalgoity, and C. Hormaeche.** 1996. Effect of interleukin 12 neutralization on host resistance and gamma interferon production in mouse typhoid. Infect. Immun. **64:**189–196.
22. **Mattner, F., J. Magram, J. Ferrante, P. Launois, K. Di Padova, R. Behin, M. Gately, J. Louis, and G. Alber.** 1996. Genetically resistant mice lacking interleukin-12 are susceptible to infection with Leishmania major and mount a polarized Th2 cell response. Eur. J. Immunol. **26:**1553–1559.
23. **McDermott, M. R., J. R. Smiley, L. J. Brais, H. E. Rudzroga, and J. Bienenstock.** 1984. Immunity in the female genital tract after intravaginal vaccination of mice with an attenuated strain of herpes simplex virus type 2. J. Virol. **51:**747–753.
24. **Milligan, G. N.** 1999. Neutrophils aid in protection of the vaginal mucosae of immune mice against challenge with herpes simplex virus type 2. J. Virol. **73:**6380–6386.
25. **Milligan, G. N., and D. I. Bernstein.** 1997. Interferon-gamma enhances resolution of herpes simplex virus type 2 infection of the murine genital tract. Virology **229:**259–268.
26. **Milligan, G. N., D. I. Bernstein, and N. Bourne.** 1998. T lymphocytes are required for protection of the vaginal mucosae and sensory ganglia of immune mice against reinfection with herpes simplex virus type 2. J. Immunol. **160:**6093–6100.
27. **O'Garra, A.** 1998. Cytokines induce the development of functionally heterogenous T helper cell subsets. Immunity **8:**275–283.
28. **Ohkusu, K., T. Yoshimoto, K. Takeda, T. Ogura, S. Kashiwamura, Y. Iwakura, S. Akira, H. Okamura, and K. Nakanishi.** 2000. Potentiality of interleukin-18 as a useful reagent for treatment and prevention of *Leishmania major* infection. Infect. Immun. **68:**2449–2456.
29. **Oxenius, A., U. Karrer, R. M. Zinkernagel, and H. Hengartner.** 1999. IL-12 is not required for induction of type 1 cytokine responses in viral infections. J. Immunol. **162:**965–973.
30. **Parr, E. L., and M. B. Parr.** 1997. Immunoglobulin G is the main protective antibody in mouse vaginal secretions after vaginal immunization with attenuated herpes simplex virus type 2. J. Virol. **71:**8109–8115.
31. **Parr, M. B., L. Kepple, M. McDermott, M. D. Drew, J. J. Bozzola, and E. L. Parr.** 1994. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. Lab. Investig. **70:**369–380.
32. **Parr, M. B., and E. L. Parr.** 1998. Mucosal immunity to herpes simplex virus type 2 in the mouse vagina is impaired by in vivo depletion of T lymphocytes. J. Virol. **72:**2677–2685.
33. **Parr, M. B., and E. L. Parr.** 1999. The role of gamma interferon in immune resistance to vaginal infection by herpes simplex virus type 2 in mice. Virology **258:**282–294.
34. **Robinson, D., K. Shibuya, A. Mui, F. Zonin, E. Murphy, T. Sana, S. B. Hartley, S. Menon, R. Kastelein, F. Bazan, and A. O'Garra.** 1997. IGIF does not drive Th1 development but synergizes with IL-12 for interferon-γ production and activates IRAK and NFκB. Immunity **7:**571–581.
35. **Satoskar, A. R., L. M. Stamm, X. Zhang, M. Okano, J. R. David, C. Terhorst, and B. Wang.** 1999. NK cell-deficient mice develop a Th1-like response but fail to mount an efficient antigen-specific IgG2a antibody response. J. Immunol. **163:**5298–5302.
36. **Schijns, V. E. C. J., B. L. Haagmans, C. M. H. Wierda, B. Kruithof, I. A. F. M. Heijnen, G. Alber, and M. C. Horzinek.** 1998. Mice lacking IL-12 develop polarized Th1 cells during viral infection. J. Immunol. **160:**3958–3964.
37. **Seth, P., W. E. Rawls, R. Duff, F. Rap, E. Adam, and J. L. Melnick.** 1974. Antigenic differences between isolates of herpesvirus type 2. Intervirology **3:**1–14.
38. **Sinigaglia, F., D. D'Ambrosio, P. Panina-Bordignon, and L. Rogge.** 1999. Regulation of the IL-12/IL-12R axis: a critical step in T-helper cell differentiation and effector function. Immunol. Rev. **170:**65–72.
39. **Szabo, S. J., S. T. Kim, G. L. Costa, X. Zhang, C. G. Fathman, and L. H. Glimcher.** 2000. A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell **100:**655–669.
40. **Takeda, K., H. Tsutsui, T. Yoshimoto, O. Adachi, N. Yoshida, T. Kishimoto, H. Okamura, K. Nakanishi, and S. Akira.** 1998. Defective NK cell activity and Th1 response in IL-18-deficient mice. Immunity **8:**383–390.
41. **Tominaga, K., T. Yoshimoto, K. Torigoe, M. Kurimoto, K. Matsui, T. Hada, H. Okamura, and K. Nakanish.** 2000. IL-12 synergizes with IL-18 or IL-1 beta for IFN-gamma production from human T cells. Int. Immunol. **12:**151–160.
42. **Tomura, M., X. Zhou, S. Maruo, H. Ahn, T. Hamaoka, H. Okamura, K. Nakanishi, T. Tanimoto, M. Kurimoto, and H. Fujiwara.** 1998. A critical role for IL-18 in the proliferation and activation of NK1.1+ CD3− cells. J. Immunol. **160:**4738–4746.
43. **Wolf, S., P. Temple, M. Kobayashi, D. Young, M. Dicig, L. Lowe, R. Dzialo, L. Fitz, C. Ferenz, and R. Hewick.** 1991. Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. J. Immunol. **146:**3074–3081.
44. **Xing, Z., A. Zganiacz, J. Wang, M. Divangahi, and F. Nawaz.** 2000. IL-12-independent Th1-type immune responses to respiratory viral infection: requirement of IL-18 for IFN-γ release in the lung but not for the differentiation of viral-reactive Th1-type lymphocytes. J. Immunol. **164:**2575–2584.