MAJOR ARTICLE

Vaccination of Rhesus Macaques with a Recombinant Measles Virus Expressing Interleukin-12 Alters Humoral and Cellular Immune Responses

Scott J. Hoffman,[1] Fernando P. Polack,[1,2] Debra A. Hauer,[1] Mahender Singh,[4,a] Martin A. Billeter,[4] Robert J. Adams,[3] and Diane E. Griffin[1]

[1]W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, Departments of [2]Pediatrics and [3]Comparative Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland; [4]Institute of Molecular Biology, University of Zurich, Zurich, Switzerland

Lack of a vaccine for infants and immunosuppression after infection are problems associated with measles virus (MV). Because interleukin (IL)–12 has been used successfully as a vaccine adjuvant and because inhibition of IL-12 expression has been associated with immunosuppression during measles, the addition of IL-12 may enhance the immune response to MV. To determine the effect of IL-12 supplementation, rhesus macaques were vaccinated with a recombinant MV expressing IL-12; these macaques had increased interferon-γ production by $CD4^+$ T cells, decreased production of IL-4, and lower levels of MV-specific immunoglobulin G4 and neutralizing antibody. Lymphoproliferative responses to mitogen were not improved. IL-12 supplementation altered the T helper type 2 bias of the immune response after MV vaccination, had a detrimental effect on the protective neutralizing antibody response, and did not improve other manifestations of immunosuppression. Reduced IL-12 levels are not the sole factor in MV-induced immunosuppression.

Two serious and interrelated problems define the adverse effects of measles virus (MV) infection in developing countries. First, measles is an immunosuppressive disease that leads to increased susceptibility to infections with other pathogens, causing deaths worldwide every year; there were 800,000 deaths attributed to measles in 2000 [1]. Second, efforts to eliminate MV are hampered by the fact that the current live attenuated MV vaccine is poorly immunogenic in infants as a result of interference of transplacentally acquired maternal antibody with vaccine viral replication and immaturity of the infants' immune system [2, 3]. These problems may be linked, as suggested by the increased mortality observed after administration of high-titer, live attenuated MV vaccines to infants in the 1990s. High-titer vaccines overcame interference by transplacental antibody and immune immaturity, eliciting stronger antibody responses than regular vaccine, but may have led to immunosuppression similar to that of wild-type infection and increased mortality in female vaccine recipients [4].

MV-induced immunosuppression is characterized by transient lymphopenia during the viremic period [5], prolonged inhibition of delayed-type hypersensitivity responses in vivo [6, 7], decreased lymphocyte proliferation to mitogens in vitro [8, 9], and polarization of cytokine production toward type 2 (Th2) cytokines (e.g., interleukin [IL]–4 [10]), which is associated with a decrease in the type 1 (Th1)–inducing cytokine IL-12 [11, 12]. In fact, reduced IL-12 production by MV-infected antigen-presenting cells has been proposed as a factor that leads to Th2 cytokine skewing and immunosuppression [13]. IL-12 is important in stimulating Th1 $CD4^+$ T cell responses [14], and its deficiency

Received 13 January 2003; accepted 2 June 2003; electronically published 28 October 2003.

Financial support: National Institutes of Health (grant AI35149); Bill and Melinda Gates Foundation.

[a] Present affiliation: St. Michael's Medical Center, Hamden, Connecticut.

Reprints or correspondence: Dr. Diane E. Griffin, W. Harry Feinstone Dept. of Molecular Microbiology and Immunology, Johns Hopkins Bloomberg School of Public Health, 615 N. Wolfe St., Baltimore, MD 21205 (dgriffin@jhsph.edu).

The Journal of Infectious Diseases 2003; 188:1553–61

0022-1899/2003/18810-0019$15.00

leads to increased susceptibility to infections known to affect patients with measles during convalescence [15, 16].

IL-12 has also been shown to affect antibody production either directly, by interaction with B cells, or indirectly, by the stimulation of T helper cells [17–19], and to promote interferon (IFN)–γ production by $CD8^+$ T cells [20]. Consequently, IL-12 has been proposed for use as an adjuvant for a variety of experimental vaccines [21–24] and is particularly attractive for use with live MV vaccines, because the addition of IL-12 during administration of the live attenuated vaccine may overcome the problems posed by maternal antibody interference and immaturity of the immune system in infants while preventing the immunosuppression associated with high-titer vaccines.

Therefore, we engineered a recombinant MV vaccine strain that stably expressed human IL-12 (functional in macaques [25]) as the biologically active heterodimer p70 [26]. Rhesus macaques were vaccinated with this virus and studied to determine whether IL-12 supplementation could serve as an adjuvant for MV vaccination and/or abolish measles-associated immunosuppression. Immune responses were compared with those of monkeys vaccinated with the parent MV not expressing IL-12 (MVtag).

MATERIALS AND METHODS

Virus. Recombinant MV expressing human IL-12 (MVIL-12) was constructed from cloned Edmonton B DNA (MVtag), as described elsewhere [26]. MVIL-12 contains an extra transcription element, located between the H and large polymerase (L) genes of MV, that consists of the genes for the p40 and p35 subunits of human IL-12 divided by an internal ribosome entry site element from encephalomyocarditis virus (figure 1). MVIL-12 was grown and assayed by plaque formation in Vero cells.

Animals and vaccination. Seven MV-seronegative rhesus macaques, 1–2 years old, were obtained from the Johns Hopkins University primate facility. A total of 3×10^4 pfu of MVIL-12 (4 monkeys) or MVtag (3 monkeys) were injected intramuscularly. At 14 months after vaccination, each of the monkeys was challenged with 10^4 $TCID_{50}$ of the wild-type Bilthoven MV strain (a gift from A. Osterhaus, Erasmus University, Rotterdam). Monkeys were chemically restrained with ketamine (1 mg/kg) during all procedures.

All animal studies had the approval of and followed the guidelines of the Johns Hopkins University Animal Care and Use Committee.

Reverse-transcriptase polymerase chain reaction (RT-PCR) and Southern-blot analyses. Total cellular RNA was extracted from 1×10^6 peripheral blood mononuclear cells (PBMCs) that had been stimulated for 48 h with phytohemagglutinin (4 μg/mL PHA-P) using RNAStat 60 (Teltest) and was resuspended in 20 μL of RNase-free water. A total of 15 μL of RNA was converted into cDNA and amplified during a 1-step RT-PCR (Invitrogen) by use of N-specific primers (5′-CATTACATCAGGATCCGG-3′ and 3′-CTACTCCGCCTGGTTATG-5′). After 40 cycles, 15 μL of the PCR product was separated by 1% agarose gel electrophoresis. The PCR product was transferred overnight onto a nylon membrane (Hybond-N; Amersham Pharmacia) and was visualized by Southern-blot analysis by use of an internal N-specific digoxigenin-labeled probe (/5 Dig N/5′-GAGCCATCAGAGGAATCA-3′). Water was used as a negative control. The positive control was RNA obtained from MVtag virus grown in Vero cells. Relative amounts of N product were assessed by densitometry with the LAS-1000 Plus CCD camera system and Image Gauge software (both from Fujifilm). The lower limit of detection was 10 pg of MV N-gene RNA.

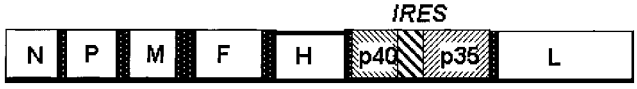

Figure 1. Measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12). The MV genome and IL-12 genes comprising the MVIL-12 virus are as follows: F, fusion; H, hemagglutinin; L, large polymerase protein of MV; M, matrix; N, nucleocapsid; P, phosphoprotein; and the p40 and p35 subunits of IL-12 separated by the internal ribosome entry site (IRES) sequences of encephalomyocarditis virus [26].

Antibody assays. EIAs to measure levels of MV-specific IgG, IgG1, IgG2, and IgG4 were performed by use of lysates of MV-infected Vero cells as antigen. H- and F-specific EIAs were performed by use of lysates of L cells expressing either the H or F glycoproteins (provided by T. F. Wild, INSERM, Lyon, France), as described elsewhere [27, 28]. IgG subclasses were measured by using monoclonal antibodies (MAbs) against human IgG1, IgG2, and IgG4 (Binding Site). Neutralizing antibody was measured by the ability of serially diluted plasma to reduce plaque formation of MV on Vero cells by 50%, as described elsewhere [29]. All assays were run in triplicate, and the results were averaged. EIA values are expressed as the difference in absorbance between MV-specific immune serum and nonimmune control serum.

Isolation of PBMCs and flow cytometry. PBMCs were separated from heparinized blood by density centrifugation on a Ficoll-Hypaque gradient (density, 1.077; Amersham). For intracellular cytokine staining, 10^6 PBMCs were incubated with 60 ng/mL PMA (Sigma), 500 ng/mL ionomycin (Sigma), and 5 μL of GolgiStop (BD PharMingen) for 5 h, blocked with 100 μL of 20% monkey serum in RPMI 1640 medium, and incubated for 15 min with 25 μg/mL fluorescein isothiocyanate–conjugated mouse anti–human CD3 (clone SP34; BD PharMingen) and Cy-Chrome–conjugated mouse anti–human CD8 (clone RPA-T8; BD PharMingen) MAb or control mouse immunoglobulin (clone MOPC-21; BD PharMingen). A phycoerythrin-conjugated mouse anti–human IFN-γ MAb (clone

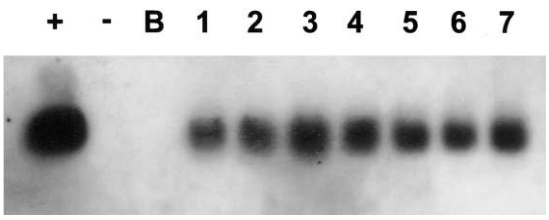

Figure 2. Reverse-transcriptase polymerase chain reaction and Southern-blot detection of measles virus (MV) in monkeys vaccinated with MV expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). *Lanes 1–4,* N-gene RNA present in peripheral blood mononuclear cells (PBMCs) derived from MVIL-12–infected monkeys; *lanes 5–7,* N-gene RNA present in PBMCs derived from MVtag-infected monkeys. +, Positive control; −, negative control; B, blank (lane skipped).

B27; BD PharMingen), control mouse immunoglobulin (clone MOPC-21; BD PharMingen), or biotinylated anti–monkey IL-4 (BioSource International), followed by phycoerythrin-conjugated streptavidin (BioSource) was used to stain intracellularly. All antibodies were used at saturating concentrations, as defined by the manufacturer. Lymphocyte acquisition was performed with a FACScalibur flow cytometer, and data were analyzed by CellQuest software (both from Becton Dickinson Immunocytometry Systems).

Lymphoproliferation and cytokine assays. PBMCs were suspended at a concentration of 10^6 cells/mL in RPMI 1640 medium supplemented with 10% fetal bovine serum. Spontaneous and PHA-induced proliferation of PBMCs were measured after 72 h of culture, as described elsewhere [8, 9]. Data are expressed in terms of the stimulation index (i.e., the ratio of PHA-induced proliferation to spontaneous proliferation). Supernatant fluids from PHA-stimulated PBMCs were collected after 48 h, and levels of IFN-γ and IL-4 were measured by EIA, according to the manufacturer's instructions (BioSource). In addition, supernatant fluids from PBMCs stimulated with 1 μg/mL *Salmonella typhimurium* lipopolysaccharide (LPS; Sigma) and 300 IU/mL IFN-γ (BD PharMingen) were collected after 24 h, and levels of IL-12 and IL-10 were measured by EIA (BioSource). IL-12 levels in plasma were also measured by EIA (BioSource).

Granzyme B assay. Cytotoxic T lymphocyte (CTL) activity was assessed by measuring granzyme B activity in PBMCs, as described elsewhere [30, 31].

Statistical analysis. Data between groups immunized with either MVIL-12 or MVtag were compared by an unpaired Student's *t* test by Stat-View software (version 5.0.1; SAS Institute). For plasma IL-12 levels, data from the MVIL-12 and MVtag groups after vaccination were compared with baseline data from

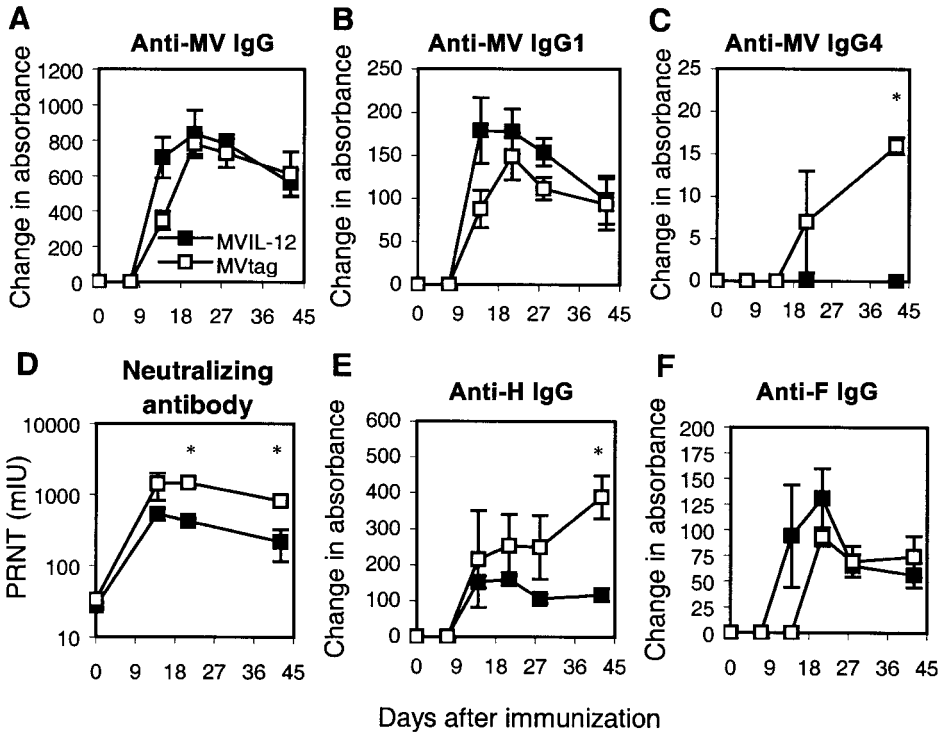

Figure 3. Measles virus (MV)–specific antibodies produced by monkeys vaccinated with MV expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). MV-specific IgG against total MV protein *(A),* H glycoprotein *(E),* and F glycoprotein *(F),* as measured by EIA. *B,* MV-specific IgG1. *C,* MV-specific IgG4. *D,* MV-neutralizing antibody, as measured by plaque reduction. Symbols indicate geometric mean titers. Error bars indicate SE. $*P < .05$. PRNT, plaque reduction neutralization titer.

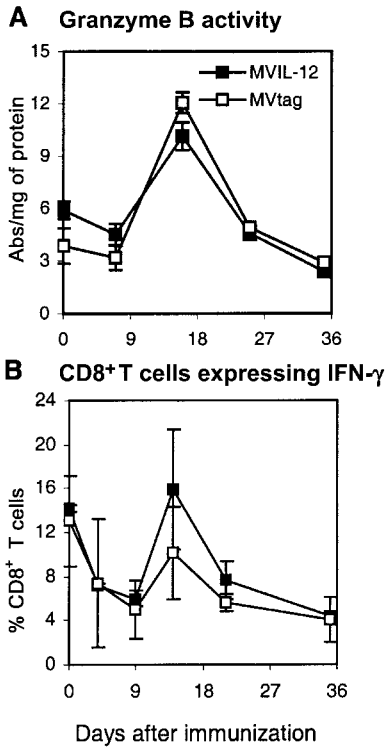

Figure 4. Cytotoxic T lymphocyte response and $CD8^+$ T cell activity in peripheral blood mononuclear cells from monkeys vaccinated with measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). *A,* Granzyme B activity reported as absorbance (Abs) per milligram of total cellular protein. *B,* Change in the percentages of PMA/ionomycin-stimulated $CD3^+/CD8^+$ T cells expressing intracellular interferon (IFN)–γ after vaccination. Error bars indicate SE.

the MVIL-12 and MVtag groups, respectively, by an unpaired Student's *t* test.

RESULTS

Use of IL-12 as an Adjuvant for MV Vaccination

Infectivity of MVIL-12 and MV-tag in macaques. Although replication is delayed by 12 h, MVIL-12 grows to titers comparable to that of the parent MV in Vero cells [26]. To confirm MVIL-12 replication in vivo, we assessed the relative amounts of MV RNA in PBMCs by use of RT-PCR and Southern-blot analysis. N-gene RNA in PBMCs was present in comparable levels on day 7 in monkeys vaccinated with MVIL-12 and MVtag virus (figure 2) but it was not detectable in PBMCs obtained from any of the monkeys on day 0 or day 25 (data not shown).

MV-specific antibody production after vaccination with MVIL-12 and MVtag. All monkeys showed an increase in MV-specific IgG that was first detected 14 days after vaccination (figure 3*A*). Both MVIL-12 and MVtag recipients had detectable MV-neutralizing antibody by day 14. However, neutralizing antibody titers were significantly higher in MVtag-vaccinated monkeys than MVIL-12–vaccinated monkeys (day 21, $P = .035$; day 42, $P = .02$; figure 3*D*). Neutralizing antibodies against MV are directed against both surface glycoproteins, H and F. To determine whether the lower neutralization titers in MVIL-12–vaccinated monkeys reflected an altered response to one or both glycoproteins, H- and F-specific EIAs were performed. Levels of IgG to H, the major neutralizing antigen, were higher in the MVtag- than MVIL-12–vaccinated monkeys (day 42, $P = .01$; figure 3*E*). However, levels of IgG to the minor neutralizing antigen F were higher and detected earlier in MVIL-12–vaccinated monkeys (figure 3*F*).

To further characterize the nature of the antibody responses, we examined the isotypes and IgG subclasses. IgG1 was the primary IgG subclass produced in both groups but was present at higher levels in MVIL-12–vaccinated monkeys than in MVtag-vaccinated monkeys (figure 3*B*). MV-specific IgG4 (day 42, $P = .0001$) was detectable only in MVtag-vaccinated mon-

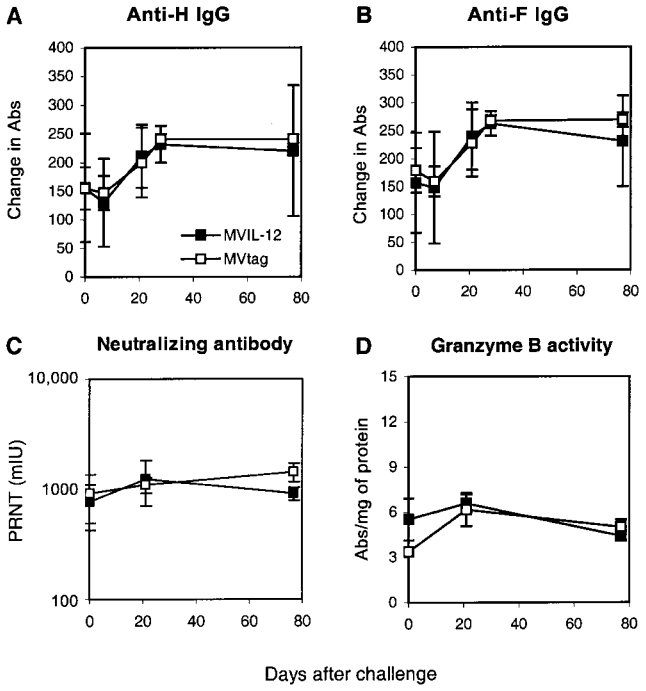

Figure 5. Antibody and cytotoxic T lymphocyte responses to challenge with wild-type virus in monkeys vaccinated with measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). MV-specific IgG against H glycoprotein *(A)* and F glycoprotein *(B),* as measured by EIA. *C,* MV-neutralizing antibody, as measured by plaque reduction. *D,* Granzyme B activity reported as absorbance (Abs) per milligram of total cellular protein. Symbols indicate geometric mean titers. Error bars indicate SE. PRNT, plaque reduction neutralization titer.

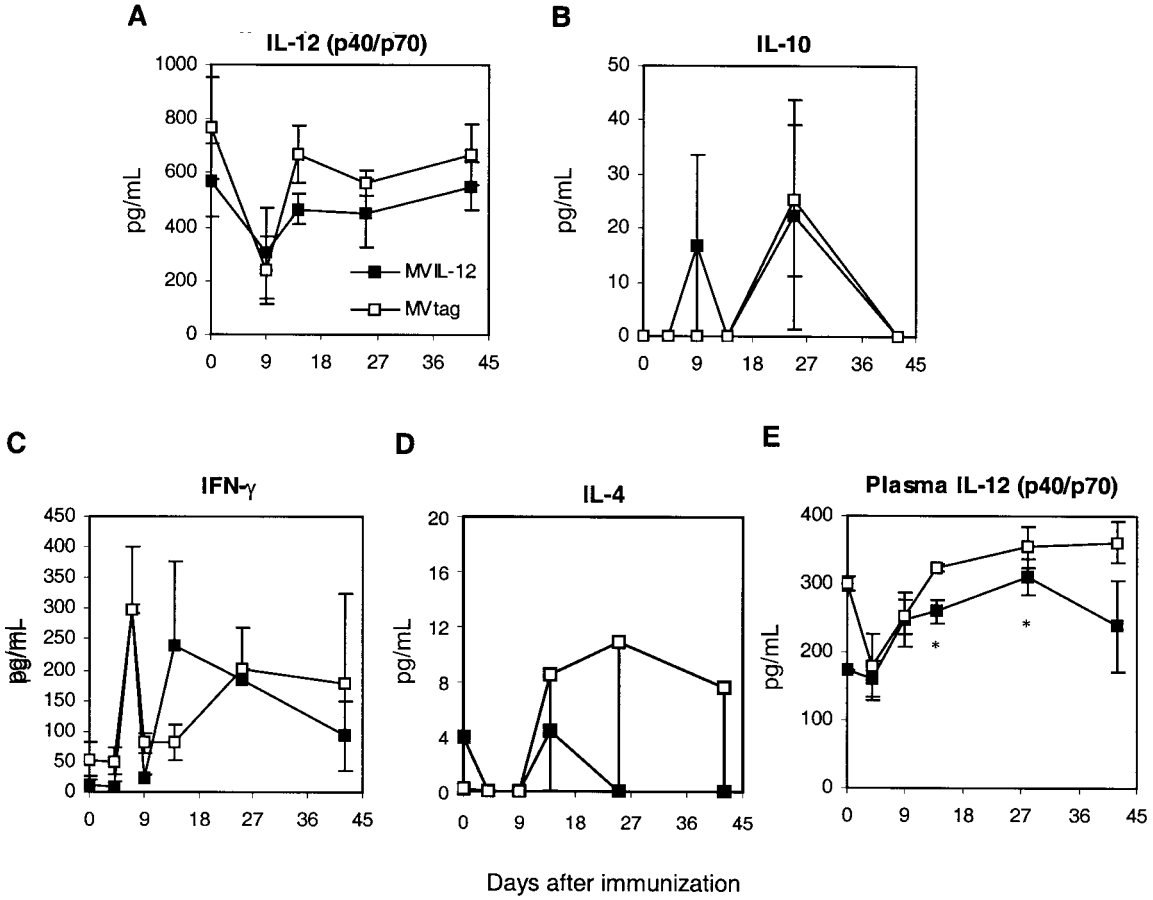

Figure 6. Cytokines secreted by peripheral blood mononuclear cells (PBMCs) from macaques and in plasma at various times after vaccination with measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). Cytokine levels were measured by EIA in supernatant fluids of lipopolysaccharide /interferon (IFN)–γ– or PHA-stimulated PBMCs after infection with MVIL-12 or MVtag, *A,* IL-12; *B,* IL-10; *C,* IFN-γ; *D,* IL-4; and *E,* IL-12 levels in plasma. Error bars indicate SE. *$P < .05$, day 14 and day 28 IL-12 levels vs. baseline IL-12 level (day 0) in MVIL-12–infected monkeys.

keys (figure 3*C*). MV-specific IgG2 and IgE were undetectable in both groups (data not shown).

CTL responses and CD8⁺ T cell activity after vaccination with MVIL-12 and MVtag. Because IL-12 enhances the lytic capacity of CTLs, effector cells that assist in the clearance of MV-infected cells [32], we measured granzyme B activity, a correlate of CTL activity [30, 31], in extracts from PBMCs at various times after vaccination (figure 4*A*). Granzyme B activity decreased slightly in both groups during the first week after vaccination (correlating with the lymphopenia) and peaked by the end of the second week. The addition of IL-12 did not augment this response.

$CD8^+$ T cells were stained with fluorochrome-labeled antibodies against CD8 and CD3 surface markers for specificity. Because IL-12 also increases production of IFN-γ by T cells [20], which might help mediate an effective Th1 response against MV, intracellular IFN-γ levels were assessed, by use of flow cytometry, in stimulated PBMCs derived from MVIL-12– and MVtag-vaccinated monkeys. Like granzyme B activity, the percentage of $CD8^+$ T cells producing IFN-γ initially decreased in both groups and then peaked on day 14 (figure 4*B*). A higher percentage of $CD8^+$ T cells from MVIL-12–vaccinated macaques were IFN-γ positive, than from MVtag-vaccinated monkeys, but the difference was not statistically significant.

Challenge of macaques vaccinated with MVIL-12 and MVtag with wild-type MV. To determine whether vaccination with supplemental IL-12 affected protection from challenge with wild-type virus, monkeys were challenged 14 months after vaccination with a strain and dose of wild-type MV known to produce measles in macaques [27, 28]. All macaques in both groups were protected against MV challenge. None of the monkeys developed a rash or had evidence of viremia (data not shown). The neutralizing antibody response after challenge remained stable, which confirms the protective effect of vaccination (figure 5*C*). No significant changes or differences were observed in anti-H or anti-F antibody responses between

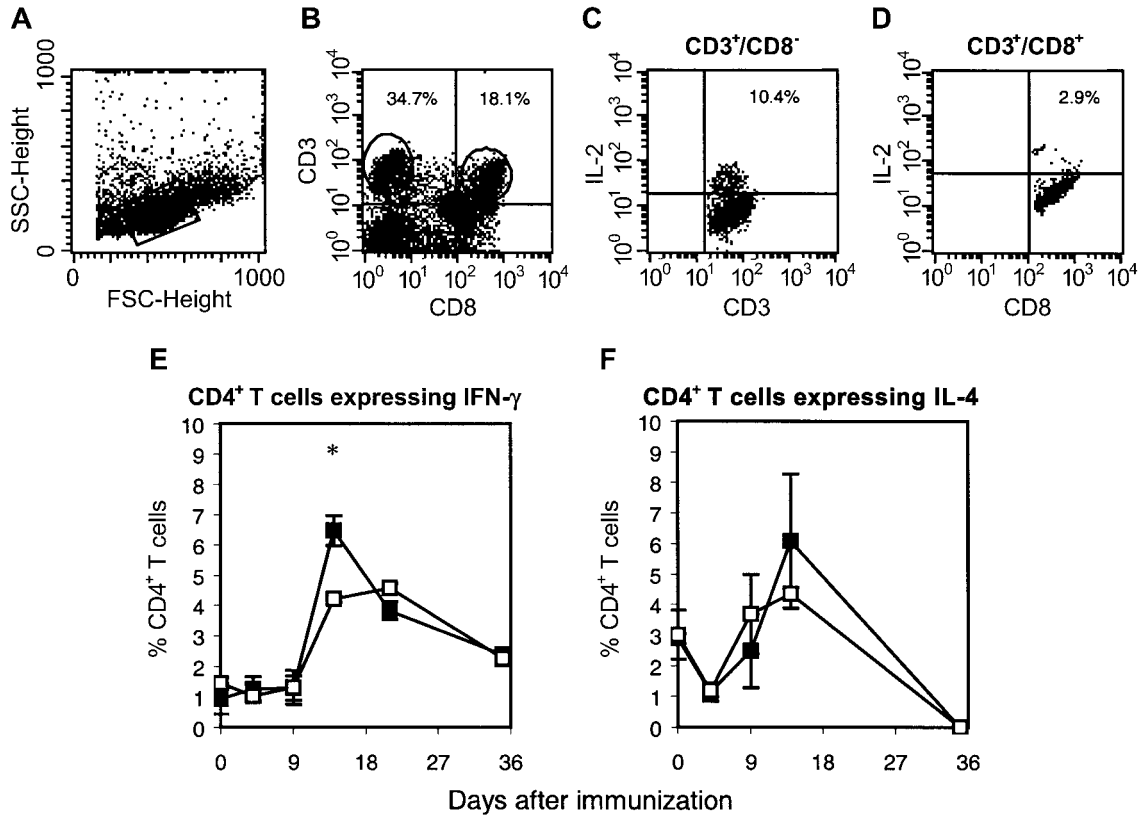

Figure 7. Flow cytometric analysis of cytokine production in monkeys after vaccination with measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). *A,* Forward scatter (FSC) and side scatter (SSC) plot of peripheral blood mononuclear cells obtained from an MVtag-vaccinated monkey, after stimulation with PMA and ionomycin. *B,* Percentage of gated lymphocytes staining positive for CD3 antibody alone, and CD3 and CD8 antibodies combined. *C,* Percentage of $CD3^+/CD8^-$ (CD4) T cells expressing intracellular IL-2 on day 10. *D,* Percentage of $CD3^+/CD8^+$ (CD8) T cells expressing intracellular IL-2 on day 10. Time course of PMA/ionomycin-stimulated $CD3^+/CD8^-$ T cells expressing intracellular interferon (IFN)–γ *(D)* and IL-4 *(E)* in or MVtag- or MVIL-12–infected monkeys. Error bars indicate SE. $*P = .05$.

groups, and these responses correlated with the neutralizing antibody response (figure 5*A* and 5*B*). CTL responses were similar in both groups (figure 5*D*).

IL-12 Supplementation to Prevent MV-Associated Immunosuppression

Cytokine secretion after vaccination with MVIL-12 and MVtag. To examine the influence of supplemental IL-12 on cytokine production, supernatant fluids of stimulated PBMCs were assayed at various times after vaccination (figure 6). IL-12 levels in supernatant fluids of PBMCs stimulated with IFN-γ and LPS were not affected by IL-12 supplementation and were identical in both groups, whereas IL-10 increased in both groups during convalescence (figure 6*A* and 6*B*). However, plasma IL-12 levels in MVIL-12–vaccinated monkeys were significantly higher (day 14, $P = .036$; day 28, $P = .035$), compared with baseline levels (MVIL-12, day 0), whereas plasma IL-12 levels in MVtag-vaccinated monkeys did not change from baseline (figure 6*E*).

IFN-γ production in supernatant fluids of PHA-stimulated PBMCs was maximal on day 7 in both groups (probably associated with NK cell activity) and increased again on day 14 only in MVIL-12–vaccinated monkeys (figure 6*C*). IL-4 was detected more often in supernatant fluids of MVtag-vaccinated macaques (figure 6*D*).

Flow cytometric analysis of $CD4^+$ T cell cytokine production after vaccination with MVIL-12 and MVtag. To assess intracellular cytokine production in T cells after vaccination with the 2 viruses, PBMCs from both groups of monkeys were stimulated with PMA and ionomycin and stained for intracellular IFN-γ and IL-4. Because macrophages can express CD4 and because NK cells can express CD8 in monkeys [33], $CD4^+$ and $CD8^+$ T cells were identified during flow cytometric analysis by staining for both CD3 and CD8 (figure 7). Cells in the gated lymphocyte population (based on forward and side scatter properties), which stained brightly with the CD8 antibody and were positive for CD3 ($CD3^+/CD8^+$), were identified as CD8 T cells, and lymphocytes that were positive for CD3 and negative for CD8 ($CD3^+/CD8^-$) were identified as CD4 T cells (figure 7*A* and 7*B*). This method for identifying individual T

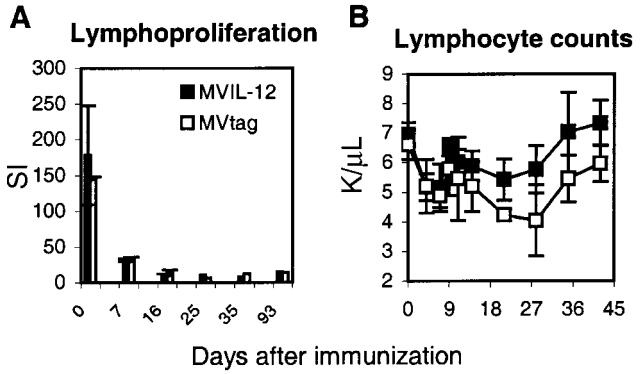

Figure 8. Changes in lymphoproliferation and lymphocyte counts after vaccination with measles virus (MV) expressing human interleukin (IL)–12 (MVIL-12) or parent MV not expressing IL-12 (MVtag). *A,* Proliferation of peripheral blood mononuclear cells after 72 h of culture, reported as the stimulation index (SI) (i.e., the ratio of phytohemagglutinin-induced proliferation to spontaneous proliferation). *B,* Lymphocyte counts. Error bars indicate SE.

cell subsets provided a CD4:CD8 T cell ratio of ~1.5:2, which is comparable to that in humans. The restricted populations were subsequently gated to evaluate cytokine production (figure 7*C* and 7*D*).

$CD4^+$ T cells from both groups of monkeys increased production of IL-4 on day 9 (figure 7*F*) and decreased below baseline levels by day 35. The percentage of $CD4^+$ T cells producing IFN-γ was significantly greater in the MVIL-12–vaccinated monkeys than in MVtag-vaccinated monkeys during the period of viremia and, thus, during the period of MVIL-12 expression leading to IL-12 production ($P = .05$; figure 7*E*).

Lymphocyte proliferation after vaccination with MVIL-12 and MVtag. To determine the effect of IL-12 on the functional responsiveness of T lymphocytes after vaccination, we measured the proliferation of PHA-stimulated and -unstimulated PBMCs from MVIL-12– and MVtag-vaccinated monkeys (figure 8*A*). Both groups exhibited a profound decrease in lymphoproliferative responses to mitogen on day 7, concurrent with lymphopenia (figure 8*B*). Decreased proliferation persisted for at least 14 weeks and was not different between the 2 groups.

DISCUSSION

In the present study, we have examined the role of IL-12 as an adjuvant in MV vaccination and as a supplement to prevent MV-associated immunosuppression. Supplemental IL-12 led to an increased percentage of CD4 T cells producing IFN-γ but had no effect on the CTL response or on the suppression of lymphoproliferative responses after MV vaccination. Furthermore, supplemental IL-12 decreased the levels of neutralizing and H-specific antibodies induced by MV vaccination. However, it did not abrogate vaccine-induced protection from challenge with wild-type MV.

IL-12 promoted a Th1 bias in the overall immune response, because CD4 T cells were more likely to produce IFN-γ in the MVIL-12–vaccinated macaques than in the MVtag-vaccinated macaques. In addition, fewer monkeys in the MVIL-12 group than in the MVtag group had detectable IL-4 in supernatant fluids of stimulated PBMCs. This was reflected in higher levels of IgG1 and a lack of production of IgG4 in MVIL-12–vaccinated monkeys. Interestingly, the specificity of the antibody response to MV glycoproteins was altered after vaccination so that MVIL-12–vaccinated monkeys had an increased response to the F protein and a decreased response to the H protein, compared with that of MVtag-vaccinated monkeys. This probably accounted for the decreased production of neutralizing antibodies after MVIL-12 vaccination, because antibodies against H are more potent at neutralizing MV than those against F [28]. A similar glycoprotein-specific effect was evident in recipients of a DNA vaccine against respiratory syncytial virus (RSV), where the addition of IL-12 decreased the RSV attachment glycoprotein (G)–specific antibody response and improved the RSV F-specific response [34]. This suggests that production of antibody to MV H or RSV G is more dependent on Th2 cytokines than is production of antibody to F.

Suppression of IL-12 production by macrophages and dendritic cells, as observed in previous studies [13, 35], has been postulated to play a role in the Th2 skewing of the cytokine profile during MV infection. Altered IL-12 synthesis by neutrophils, another source of immunomodulatory cytokines, may also be involved in the switch to Th2 cytokine production during measles [11]. The MVIL-12 virus was designed, in part, with the intent of determining whether the restoration of IL-12 production could ameliorate immunosuppression during MV infection, which includes the inhibition of delayed-type hypersensitivity responses in vivo and a decrease in lymphocyte proliferation to mitogens in vitro.

The present study has shown that IL-12 may not be sufficient to correct the deficit in T cell proliferative responses to mitogens observed in vitro after MV infection, a finding that confirms previous observations by Gans et al. [36] and that may be explained by a new and interesting observation potentially associated with MV immunosuppression—a sustained increase in IL-10 levels during convalescence [37]. IL-10 is an immunomodulatory cytokine known to have similar effects as MV on autoimmune diseases and latent infections, such as Crohn disease, nephrotic syndrome, and juvenile rheumatoid arthritis [38–40].

The specific levels of IL-12 produced in MVIL-12– and MVtag-vaccinated monkeys are unknown, although the amount of circulating IL-12 was generally higher (compared with baseline) in the MVIL-12–vaccinated monkeys than the MVtag-vaccinated

monkeys. The p40 and p35 genes of IL-12 were inserted between the H and L genes of MVtag to avoid excessive IL-12 production. MV transcribes genes sequentially, resulting in a gradient of mRNAs and thus virally expressed proteins. Consequently, gene products, such as p40 and p35, in MVIL-12 are not produced in as large quantities as are more upstream gene products. Nonetheless, changes in the humoral and cellular responses indicate that IL-12 levels in MVIL-12–vaccinated monkeys were sufficient to have a biologic effect. Therefore, it is unlikely that further increasing the production of IL-12 during MV replication would provide benefit.

In summary, use of an MVIL-12 recombinant virus affected the specificity and biological activity of the antibody response to MV by decreasing neutralizing antibody responses, anti-H antibody production, and the MV-specific IgG4 response while increasing MV-specific anti-F responses. These effects were associated with an alteration of the Th2 bias of the immune response typically associated with MV infection. Manifestations of MV-associated immunosuppression, such as decreased in vitro lymphoproliferative responses and lymphopenia, remained unaffected, suggesting that IL-12 suppression is not the sole factor in MV-induced immunosuppression.

Acknowledgments

We thank William J. Moss, for assisting with the statistical analysis, and Christopher Karp, for helpful discussions.

References

1. Global measles mortality reduction and regional elimination, 2000–2001. Part II. Wkly Epidemiol Rec **2002**; 77:58–61.
2. Gans HA, Arvin AM, Galinus J, Logan L, DeHovitz R, Maldonado Y. Deficiency of the humoral response to measles vaccine in infants immunized at age 6 months. JAMA **1998**; 280:527–32.
3. Albrecht P, Ennis FA, Saltzman EJ, Krugman S. Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. J Pediatr **1977**; 91:715–18.
4. Ryon JJ, Moss WJ, Monze M, Griffin DE. Functional and phenotypic changes in circulating lymphocytes from hospitalized Zambian children with measles. Clin Diagn Lab Immunol **2002**; 9:994–1003.
5. Moss WJ, Polack FP. Immune responses to measles and measles vaccine: challenges for measles control. Viral Immunol **2001**; 14:297–309.
6. Pirquet CV. Das verhalten der kutanen Tuberkulin-reaktion wahrend der Masern. Dtsch Med Wochenschr **1908**; 30:1297–300.
7. Tamashiro VG, Perez HH, Griffin DE. Prospective study of the magnitude and duration of changes in tuberculin reactivity during uncomplicated and complicated measles. Pediatr Infect Dis J **1987**; 6:451–4.
8. Ward BJ, Johnson RT, Vaisberg A, Jauregui E, Griffin DE. Spontaneous proliferation of peripheral mononuclear cells in natural measles virus infection: identification of dividing cells and correlation with mitogen responsiveness. Clin Immunol Immunopathol **1990**; 55:315–26.
9. Ward BJ, Johnson RT, Vaisberg A, Jauregui E, Griffin DE. Cytokine production in vitro and lymphoproliferative defect of natural measles virus infection. Clin Immunol Immunopathol **1991**; 61:236–48.
10. Ward BJ, Griffin DE. Differential CD4 T cell activation in measles. J Infect Dis **1993**; 168:275–81.
11. Polack FP, Hoffman SJ, Moss WJ, Griffin DE. Altered synthesis of interleukin-12, type 1 and type 2 cytokines in rhesus macaques during measles and atypical measles. J Infect Dis **2002**; 185:13–9.
12. Atabani SF, Byrnes AA, Jaye A, et al. Natural measles causes prolonged suppression of interleukin-12 production. J Infect Dis **2001**; 184:1–9.
13. Karp CL, Wysocka M, Wahl LM, et al. Mechanism of suppression of cell-mediated immunity by measles virus. Science **1996**; 273:228–31.
14. Trinchieri G. Immunobiology of interleukin-12. Immunol Res **1998**; 17:269–78.
15. Haraguchi SK, Day NK, Nelson RP Jr, et al. Interleukin 12 deficiency associated with recurrent infection. Proc Natl Acad Sci USA **1998**; 95: 13125–29.
16. Altare F, Lammas D, Revy P, et al. Inherited interleukin 12 deficiency in a child with bacille Calmette-Guerin and *Salmonella enteritidis* disseminated infection. J Clin Invest **1998**; 102:2035–40.
17. Skok J, Poudrier J, Gray D. Dendritic cell–derived IL-12 promotes B cell induction of Th2 differentiation: a feedback regulation of Th1 development. J Immunol **1999**; 163:4284–91.
18. Caspi RR. IL-12 in autoimmunity. Clin Immunol Immunopathol **1998**; 88:4–13.
19. Gagro A, Gordon J. The interplay between T helper subset cytokines and IL-12 in directing B lymphocyte differentiation. Eur J Immunol **1999**; 29:3369–79.
20. Trinchieri G. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol **2003**; 3:133–46.
21. Boretti FS, Leutenegger CM, Mislin C, et al. Protection against FIV challenge infection by genetic vaccination using minimalistic DNA constructs for FIV env gene and feline IL-12 expression. AIDS **2000**; 14:1749–57.
22. Hancock GE, Smith JD, Heers KM. The immunogenicity of subunit vaccines for respiratory syncytial virus after co-formulation with aluminum hydroxide adjuvant and recombinant interleukin-12. Viral Immunol **2000**; 13:57–72.
23. Kuwata T, Miura T, Haga T, Kozyrev I, Hayami M. Construction of chimeric simian and human immunodeficiency viruses that produce interleukin-12. AIDS Res Hum Retroviruses **2000**; 16:465–70.
24. Hanlon L, Argyle D, Bain D, et al. Feline leukemia virus DNA vaccine efficacy is enhanced by coadministration with interleukin-12 (IL-12) and IL-18 expression vectors. J Virol **2001**; 75:8424–33.
25. Hoffman SL, Crutcher JM, Puri SK, et al. Sterile protection of monkeys against malaria after administration of interleukin-12. Nat Med **1997**; 3:80–3.
26. Singh M, Billeter MA. A recombinant measles virus expressing biologically active human interleukin-12. J Gen Virol **1999**; 80:101–6.
27. Polack FP, Auwaerter PG, Lee SH, et al. Production of atypical measles in rhesus macaques: evidence for disease mediated by immune complex formation and eosinophils in the presence of fusion-inhibiting antibody. Nat Med **1999**; 5:629–34.
28. Polack FP, Lee SH, Permar S, et al. Successful DNA immunization against measles: neutralizing antibody against either the hemagglutinin or fusion glycoprotein protects rhesus macaques without evidence of atypical measles. Nat Med **2000**; 6:776–81.
29. Albrecht P, Herrmann K, Burns GR. Role of virus strain in conventional and enhanced measles plaque reduction test. J Virol Methods **1981**; 3: 251–60.
30. McElhaney JE, Pinkoski MJ, Au D, Lechelt KE, Bleackley RC, Meneilly GS. Helper and cytotoxic T lymphocyte responses to influenza vaccination in healthy compared to diabetic elderly. Vaccine **1996**; 14: 539–44.
31. McElhaney JE, Pinkoski MJ, Upshaw CM, Bleackley RC. The cell-mediated cytotoxic response to influenza vaccination using an assay for granzyme B activity. J Immunol Methods **1996**; 190:11–20.
32. Permar SR, Klumpp SA, Mansfield KG, et al. Role of $CD8^+$ lymphocytes in control and clearance of measles virus infection of rhesus monkeys. J Virol **2003**; 77:4396–400.
33. Dykhuizen M, Ceman J, Mitchen J, et al. Importance of the CD3

marker for evaluating changes in rhesus macaques CD4/CD8 T cell ratios. Cytometry **2000**; 40:69–75.
34. Bembridge GP, Rodriguez N, Garcia-Beato R, Nicolson C, Melero JA, Taylor G. DNA encoding the attachment (G) or fusion (F) protein of respiratory syncytial virus induces protection in the absence of pulmonary inflammation. J Gen Virol **2000**; 81:2519–23.
35. Fugier-Vivier I, Servet-Delprat C, Rivailler P, et al. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. J Exp Med **1997**; 186:813–23.
36. Gans HA, Maldonado Y, Yasukawa LL, et al. IL-12, IFN-γ, and T cell proliferation to measles in immunized infants. J Immunol **1999**; 162: 5569–75.
37. Moss WJ, Ryon JJ, Monze M, Griffin DE. Differential regulation of interleukin (IL)–4, IL-5, and IL-10 during measles in Zambian children. J Infect Dis **2002**; 186:879–87.
38. Madsen KL. Inflammatory bowel disease: lessons from the IL-10 gene deficient mouse. Clin Invest Med **2001**; 24:250–7.
39. Gallagher KT, Bernstein B. Juvenile rheumatoid arthritis. Curr Opin Rheumatol **1999**; 11:372–6.
40. Matsumoto K, Ohi H, Kanmatsuse K. Interleukin 10 and interleukin 13 synergize to inhibit vascular permeability factor release by peripheral blood mononuclear cells from patients with lipoid nephrosis. Nephron **1997**; 77:212–8.