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Mechanism of Measles Virus–Induced Suppression of Inflammatory Immune Responses

Julien C. Marie,*§ Jeanne Kehren,* Marie-Claude Trescol-Biémont,* Alexey Evlashev,* Hélène Valentin,* Thierry Walzer,* Rosine Tedone,* Bruce Loveland,† Jean-François Nicolas,* Chantal Rabourdin-Combe,* and Branka Horvat*‡§

*INSERM U503, CERVI
Immunobiologie Fondamentale et Clinique
Lyon, 69365
France

†The Austin Research Institute
Heidelberg, VIC 3084
Australia

Summary

Measles virus (MV) causes profound immunosuppression, resulting in high infant mortality. The mechanisms are poorly understood, largely due to the lack of a suitable animal model. Here, we report that particular MV proteins, in the absence of MV replication, could generate a systemic immunosuppression in mice through two pathways: (1) via MV-nucleoprotein and its receptor FcγR on dendritic cells; and (2) via virus envelope glycoproteins and the MV-hemagglutinin cellular receptor, CD46. The effects comprise reduced hypersensitivity responses associated with impaired function of dendritic cells, decreased production of IL-12, and the loss of antigen-specific T cell proliferation. These results introduce a novel model for testing the immunosuppressive potential of anti-measles vaccines and reveal a specific mechanism of MV-induced modulation of inflammatory reactions.

Introduction

Measles virus (MV) is responsible for an acute childhood disease that each year infects over 40 million individuals and causes the death of more than 1 million, primarily in the developing world (Murray and Lopez, 1997). The high mortality is associated with transitional MV-induced immunosuppression, enabling secondary infections that severely complicate the course of the disease (Beckford et al., 1985; Griffin et al., 1994). Measles infection is followed by the ablation of delayed-type hypersensitivity responses to tuberculin (Von Pirquet, 1908; Tamashiro et al., 1987) and impaired in vitro proliferation of peripheral blood lymphocytes (PBL) (Hirsch et al., 1984) as well as allospecific cytolytic activity (Galama et al., 1980). Many immunological alterations observed during measles infection also occur at lesser magnitude after vaccination of children using attenuated MV (Fireman et al., 1969; Hussey et al., 1996).

‡To whom correspondence should be addressed (e-mail: horvat@cervi-lyon.inserm.fr).
§Present address: INSERM U404, Immunité et Vaccination, CERVI, 21 avenue Tony Garnier, 69365, Lyon, France.

Although MV was the first pathogen reported to induce immunosuppression, documented nearly 100 years ago (Von Pirquet, 1908), the lack of a suitable small animal model has impeded progress in understanding the mechanisms of MV-induced immune abnormalities. Monkeys have been used (Auwaerter et al., 1999), but their utilization is extremely restricted by limited supply and high cost. SCID mice grafted with human PBL showed a reduced production of human IgG after MV infection (Tishon et al., 1996); nevertheless, this model is not convenient for an accurate analysis of antigen-specific responses due to the lack of MHC compatibility between murine stromal tissue and human PBL. Cotton rats, susceptible to MV infection, subsequently develop immunosuppression (Niewiesk et al., 1997) but compared to mice are genetically and immunologically poorly characterized. Recently, MV infection of mice expressing CD46, human cellular receptor of MV, was reported to be associated with immunosuppression (Oldstone et al., 1999), though several other reports suggested that MV proteins alone can inhibit in vitro immune response (Karp et al., 1996; Schlender et al., 1996; Ravanel et al., 1997). Although these studies confirmed the existence of MV-induced restraint of immune response, the importance of MV replication and the mechanism responsible for induction of immunosuppression in vivo remains elusive.

In this report, we investigated the roles of MV proteins in the generation of immunosuppression in vivo. Two types of T cell–dependent inflammatory reactions were analyzed: delayed type hypersensitivity (DTH) to keyhole limpet hemocyanin (KLH) mediated by $CD4^+$ T cells (Grabbe and Schwarz, 1998), and contact hypersensitivity (CHS) induced by epicutaneous exposure to the hapten dinitrofluorobenzene (DNFB) and mediated by $CD8^+$ T cells (Bour et al., 1995; Kehren et al., 1999). Dendritic cells play the critical role in the initiation of these inflammatory responses (Grabbe and Schwarz, 1998). We demonstrated that MV replication is not necessary for the induction of immunosuppression and that inactivated MV efficiently suppresses hypersensitivity responses in mice, after the interaction of viral envelope proteins and nucleoprotein with their respective cellular receptors. These results suggest that a conventional murine hypersensitivity model could be directly applicable in testing the immunosuppressive effects of anti-measles vaccines. Furthermore, they provide evidence that MV proteins directly induce suppression of inflammatory reactions and impair dendritic cell function and have important implications in understanding the pathogenesis of MV-induced inhibition of immunity.

Results

Inactivated Measles Virus Inhibits Hypersensitivity Responses in Mice

We first analyzed whether MV particles could affect the generation of two types of cellular immunity in mice: contact hypersensitivity to DNFB and DTH to KLH.

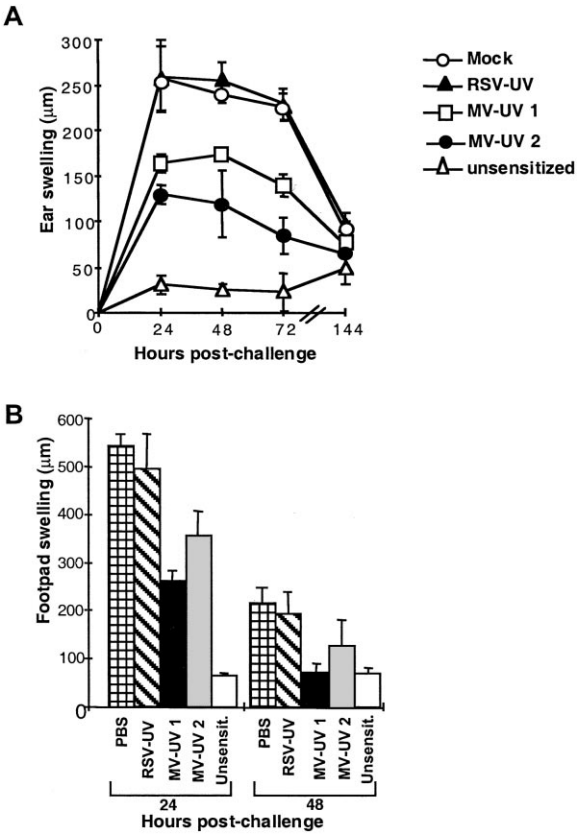

Figure 1. Effect of UV-Inactivated MV on Hypersensitivity Responses

Groups of five C57BL/6 mice were injected intraperitoneally with different concentrations of purified MV-UV: 200 μg corresponding to 5×10^6 pfu, (MV-UV1) or 40 μg corresponding to 10^6 pfu (MV-UV2), 200 μg of RSV-UV, corresponding to 5×10^6 pfu or the mock preparation.

(A) Mice were sensitized 6 hr later with DNFB or left unsensitized and challenged after 5 days with DNFB; results are expressed as mean ear swelling (± SD) at different time points after challenge.

(B) Mice were immunized 6 hr later with KLH in CFA or left unsensitized and challenged after 7 days by footpad injection of KLH in PBS. Results expressed as mean footpad swelling (± SD) are representative of three independent experiments.

C57BL/6 mice were injected intraperitoneally with different amounts of purified UV-inactivated MV (MV-UV) and sensitized 6 hr later with either DNFB on ventral skin (Figure 1A) or KLH in CFA injected subcutaneously (Figure 1B), and ear or footpad swelling, respectively, was measured after the challenge. The effect of MV was compared to another negative strand Paramyxoviridae RNA virus, respiratory syncytial virus (RSV), which infects the human respiratory system. While MV-UV significantly inhibited the inflammatory mouse ear swelling response after challenge with DNFB, injection of the same number of RSV-UV particles did not give any effect (Figure 1A), excluding the role of viral RNA. RSV-UV did not inhibit the DTH response to KLH either, but MV-UV reduced it by half (Figure 1B). In addition, similar results were obtained with purified MV-UV (Edmonston strain) and with virus supernatant, as well as with two other MV strains, Hallé and MA93F, and the inhibition of hypersensitivity response was not limited by the genetics of mice used, giving the same results with C57BL/6, BALB/c, and 129/Sv (data not shown). Finally, hypersensitivity reaction was suppressed when MV-UV was injected up to 2 days before sensitizing with the hapten (data not shown), demonstrating that inhibitory effect of MV could largely exceed 6 hr. Moreover, the use of inactivated virus excluded any infectious component to this inhibition of inflammatory responses and focused our attention on the role of viral proteins.

Effect of Measles Nucleoprotein on Hypersensitivity Responses

We next studied the MV components responsible for immunosuppression. Analysis of the MV-UV inocula by ELISA showed that the standard dose of 5×10^6 pfu of MV-UV contained 15 ± 4 μg free NP, which could participate in the inhibition of inflammatory reactions in mice. We then tested whether purified recombinant NP could bind murine dendritic cells and demonstrated an efficient binding that could be blocked by mAb specific to murine FcR (2.4G2) (Figure 2A). Similar results were obtained with murine macrophages (data not shown). As Paramixovirus nucleocapsids contain two structural units, an N-terminal domain, which interacts directly with RNA, and a C-terminal domain, which lies on the surface of the assembled nucleocapsid, we then analyzed whether the C-terminal part of NP (126 aa) could bind dendritic cells. Similarly to NP, NPc bound dendritic cells isolated from normal mice (Figure 2A) but not from gene-targeted mice lacking Fc receptor ($FcR^{-/-}$) (Figure 2B).

Mice were then injected with either purified recombinant NP or NPc and analyzed for hypersensitivity responses (Figure 2). Similarly to MV-UV, recombinant NP, as well as NPc, significantly reduced by half mouse ear swelling, indicating that this protein, particularly its C-terminal part, plays an important role in MV-induced immunosuppression (Figure 2B). The effect of NP was dose dependent: absent with 6 μg, reaching threshold with 12 μg, and maximal with 25 μg of NP (data not shown).

Both human and murine Fc receptors for IgG efficiently bind measles NP on B cells (Ravanel et al., 1997), as well as dendritic cells (Figures 2A and 2B), so we analyzed whether FcγR is directly involved in suppression. Fc receptor–deficient mice were injected intraperitoneally with either recombinant NP, NPc, or UV-inactivated MV. In contrast to C57BL/6 mice (Figure 2C) and heterozygote progeny of $FcR^{-/-}$ crossed with C57BL/6 mice (data not shown), where NP and MV-UV inhibited CHS response, in $FcR^{-/-}$ mice injection of NP, NPc, or MV-UV had no effect (Figure 2D). We next investigated whether MV-UV and its nucleoprotein could modulate DTH to KLH in wild-type and $FcR^{-/-}$ mice (Figures 2E and 2F). While both MV-UV, NP, and NPc inhibited footpad swelling in wild-type mice, this effect was again absent in $FcR^{-/-}$ mice. The immunosuppressive effect of MV proteins was equivalent to that of 1 mg of dexamethasone, which, contrary to MV-induced suppression, persisted in FcR-deficient mice, demonstrating that hypersensitivity in these mice could be suppressed by the other mechanism not requiring FcR (Figures 2E and 2F). These results indicate that an interaction of NP, particularly its C-terminal part NPc, with FcγR is critical for MV-induced immunosuppression of inflammatory responses in vivo.

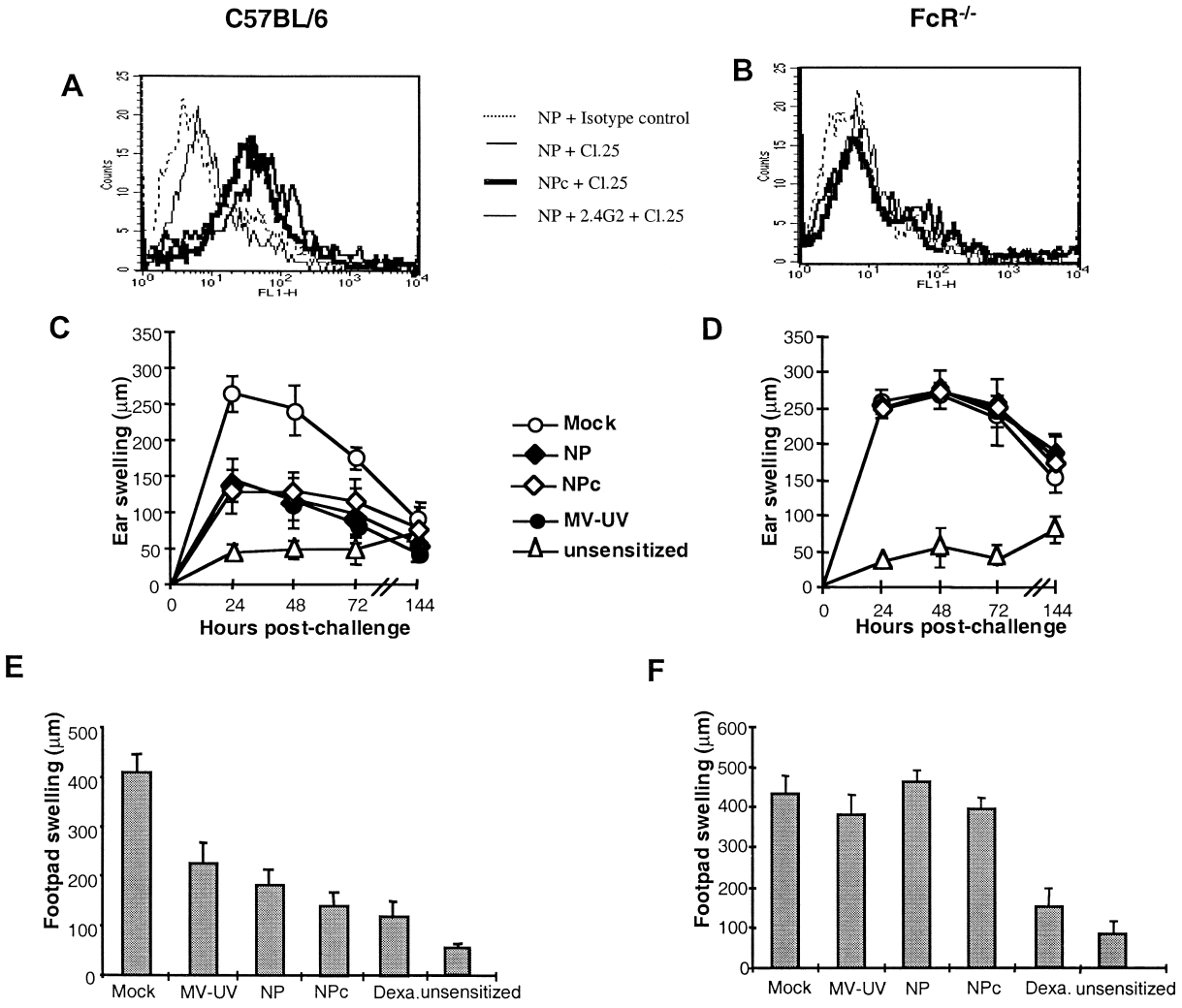

Figure 2. Measles NP Binds Dendritic Cells and Inhibits Hypersensitivity Responses

Binding of NP and NPc to DC purified from normal (A) or FcR-deficient mice (B). Cells were incubated with either NP in the absence or in the presence of anti-FcR mAb 2.4G2 or with NPc, and binding was detected with biotinylated anti-NP mAb Cl.25 and FITC-conjugated streptavidin. Mouse IgG2a-biotin was used as an isotype control to Cl.25. Hypersensitivity responses were analyzed in groups of five C57BL/6 mice (C and E) or FcR$^{-/-}$ mice (D and F), injected i.p. with the mock preparation, NP (100 μg), NPc (400 μg), 5 × 10^6 pfu of MV-UV or 1 mg of water-soluble dexamethasone (dexa).

(C–D) Mice were sensitized 6 hr later with DNFB or left unsensitized and challenged after 5 days. Results are expressed as mean ear swelling (± SD) at different time points after challenge and are representative of three independent experiments.

(E–F) Alternatively, 6 hr after MV-UV or NP injection, mice were immunized with KLH-CFA, and DTH to KLH was assessed after 7 days by footpad challenge with KLH in PBS. Footpad swelling shown as mean ± SD was measured after 24 hr and is representative of two independent experiments.

Effect of Measles Envelope Glycoproteins on CHS

The MV envelope has two glycoproteins, hemagglutinin (H) and fusion (F) protein. The human CD46 molecule, not expressed in mice, binds MV-H and is a cellular receptor for MV-Edmondston strain (Dorig et al., 1993; Naniche et al., 1993), as well as for some clinical isolates (Manchester et al., 2000). To assess the role of H and F protein in MV-induced immunosuppression, we used transgenic mice ubiquitously expressing CD46 at levels resembling those of human tissues (Thorley et al., 1997). C57BL/6 and CD46 transgenic mice were injected with UV-inactivated recombinant MV in which H and F were replaced by vesicular stomatitis G protein (MGV): its effect on the generation of CHS to DNFB was compared with wild-type UV-inactivated MV and its corresponding recombinant virus, EDtag-UV. Injection of MGV-UV reduced CHS response by half in both mouse strains (Figure 3). Similar results were obtained with 100 μg of NP (data not shown). The suppressive effect of MGV-UV was analogous to the effect of EDtag-UV and MV-UV in normal C57BL/6 mice (Figure 3A), indicating that viral H and F were not critical for the induction of suppression in the absence of CD46. However, immunosuppressive effect was not observed after injection of vesicular stomatitis virus (VSV-UV), excluding the role of VSV-G protein as well as viral RNA in the MGV-UV induced sup-

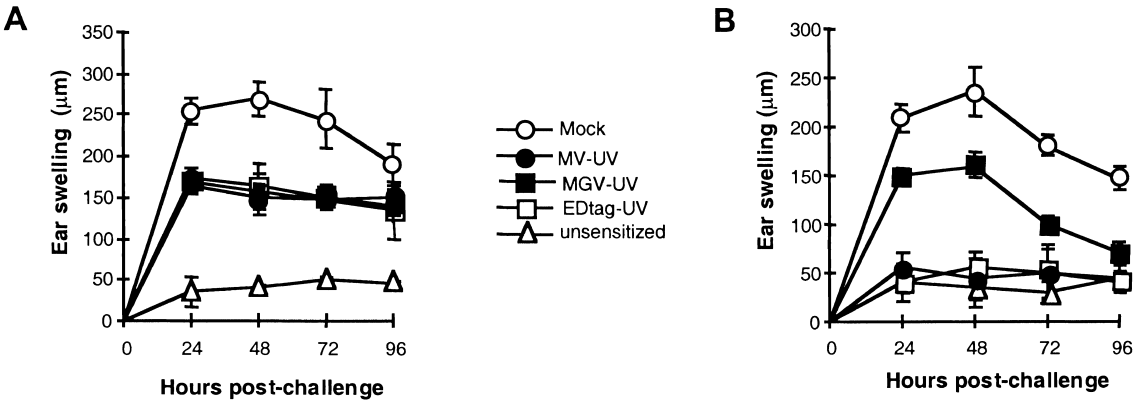

Figure 3. Effect of Measles Envelope Glycoproteins on Contact Hypersensitivity

Groups of five C57BL/6 mice (A) or CD46 transgenic mice (B) were injected with either the mock preparation or 5×10^6 pfu of UV-inactivated viruses: MV-UV, MGV-UV, or EDtag-UV, and sensitized 6 hr later with DNFB or left unsensitized. Mice were challenged after 5 days, and results expressed as mean ear swelling (± SD) at different time points after challenge are representative of two independent experiments.

pression (data not shown). Furthermore, inactivated wild-type MV and its recombinant virus EDtag entirely abolished ear swelling responses in CD46 transgenic mice (Figure 3B), demonstrating that the interaction between MV-H and CD46 significantly enhanced antiinflammatory effect.

Therapeutic Efficacy of MV Proteins

Given the prophylactic potential of MV proteins in the induction of inflammatory responses, we analyzed their effect on the effector phase of CHS. Mice were DNFB sensitized and injected 5 days later with MV-UV. The effector phase was initiated by DNFB challenge 6 hr (Figure 4A) and 2 weeks later (Figure 4B), and ear swelling was measured 24 hr after challenge. A single injection of MV-UV into sensitized C57BL/6 mice was sufficient to inhibit CHS, demonstrating that MV proteins suppress the effector phase of CHS as well. Similar to the effect observed at sensitization phase (Figure 3B), the injection of MV-UV into already sensitized CD46 transgenic mice completely inhibited ear swelling (Figure 4A), suggesting that CD46 expression increases the inhibitory effect of MV proteins. In addition, inhibition of CHS occurred in CD46 transgenic but not wild-type mice challenged with DNFB 2 weeks after MV-UV injection (Figure 4B), indicating that CD46 expression not only strengthened but also lengthened the duration of the antiinflammatory effects of MV proteins. Finally, these results indicate the important systemic therapeutic efficacy of MV proteins both in prevention and treatment of inflammatory responses.

MV Proteins Induce an Impaired Capacity of Antigen-Presenting Cells to Stimulate Antigen-Specific T Cell Proliferation

Generation of CHS depends on the activity of $CD8^+$ T cells (Bour et al., 1995; Kehren et al., 1999). Therefore, we analyzed whether CHS inhibition in MV-UV injected mice is associated with impaired priming of antigen-specific $CD8^+$ T cells in lymphoid organs. C57BL/6, CD46-transgenic, or $FcR^{-/-}$ mice were injected with MV-UV or NP and 6 hr later sensitized with DNFB. Lymphoid cells recovered from regional draining lymph nodes 4 days later were tested for hapten-specific proliferative responses (Figure 5A). $CD8^+$ T lymphocytes from mock-injected and sensitized mice responded vigorously to

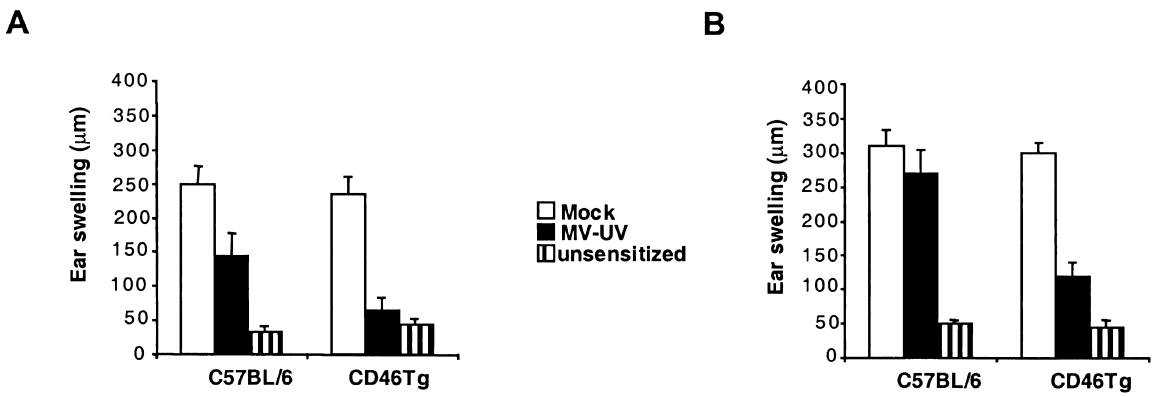

Figure 4. Therapeutic Efficacy of MV Proteins

Groups of five C57BL/6 or CD46 transgenic mice were sensitized with DNFB and injected 5 days later with either 5×10^6 pfu of MV-UV or mock, or left unsensitized. Mice were ear-challenged 6 hr (A) and 2 weeks (B) after sensitization. Results are expressed as mean ear swelling (± SD) at 24 hr after challenge.

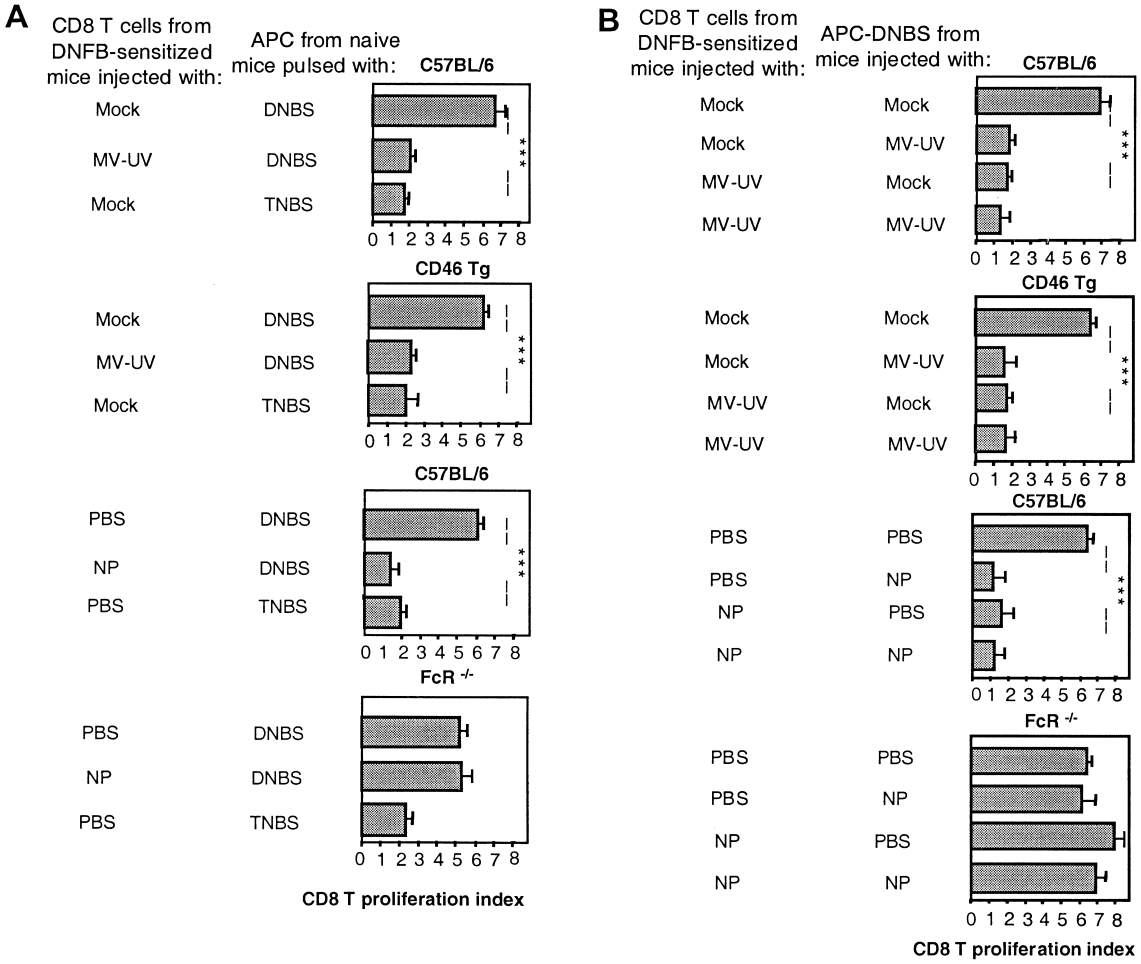

Figure 5. Impaired Capacity of APC from MV-UV- and NP-Injected Mice to Stimulate Antigen-Specific T Cell Proliferation

C57BL/6, CD46 transgenic, or FcR$^{-/-}$ mice to be used as donors of CD8$^+$ T cells were injected with either 5 $\times$ 10^6 pfu of UV-inactivated MV, the mock preparation, 100 μg of recombinant NP or PBS, as indicated. Donors of APC were either naive syngeneic mice (A) or mice that received the same treatment as donors of CD8$^+$ T cells (B). Mice were sensitized with DNFB 6 hr after injection, for donors of T lymphocytes, or left unsensitized for APC donors. Four days later, lymph nodes were collected from three mice of the same group, and cell cultures were set up to measure the DNFB-specific proliferation of CD8$^+$ T lymphocytes. Irradiated splenocytes from either mock/PBS- or MV-UV/NP-injected mice were coupled to either DNBS (A and B) or TNBS (A) and used as APC for CD8$^+$ T lymphocytes of either mock/PBS or MV-UV/NP-injected and DNFB sensitized mice. Specific proliferation responses were determined after 3 days of culture by ^{3}H-thymidine incorporation for 16 hr and against DNBS modified syngenic spleen cells varied between 25,000 and 35,000 cpm. Results are expressed as a proliferation index, and representative data of three independent experiments are shown as mean $\pm$ SD (***P $<$ 0.001, Student's T-test).

DNBS-pulsed naive syngeneic antigen-presenting cells (APC), but not to TNBS-coupled splenocytes, confirming the antigen specificity of the response. However, prior single injection of C57BL/6 or CD46 transgenic mice with either MV-UV or NP entirely abolished in vitro antigen-specific proliferation of their CD8$^+$ T cells in response to DNBS-coupled APC. Nevertheless, this inhibition did not occur in FcR$^{-/-}$ mice injected with the same dose of NP. These results demonstrate the important role of FcR in mediating the MV-induced defect in the priming of CD8$^+$ T lymphocytes in vivo.

We next investigated whether impaired APC capacity modulated the proliferation of CD8$^+$ lymphocytes primed normally in vivo. CD8$^+$ T lymphocytes obtained from mock-injected and DNFB-sensitized mice did not proliferate when cultured with hapten-pulsed splenocytes (APC) from either MV-UV or NP injected CD46-transgenic or wild-type mice (Figure 5B). These results are in agreement with the capacity of MV proteins to inhibit the effector phase of CHS (Figure 4A) and were reproduced with purified MV as well as with virus supernatant. The inhibitory effect of MV proteins was absent in FcR$^{-/-}$ mice, highlighting the importance of the interaction of NP with FcR on APC in the pathogenesis of MV-induced suppression. Altogether, these results indicate that MV-UV and particularly NP impair the function of APC via FcγR, affecting not only the priming of naive CD8$^+$ lymphocytes during sensitization but also the development of the effector phase following secondary antigen contact.

MV-UV Decreases the Percentage of IL-12-Producing Dendritic Cells in Regional Lymph Nodes

APC essential for priming of hypersensitivity responses are dendritic cells (DC) (Grabbe and Schwarz, 1998). As DC have been identified as the major producers of IL-

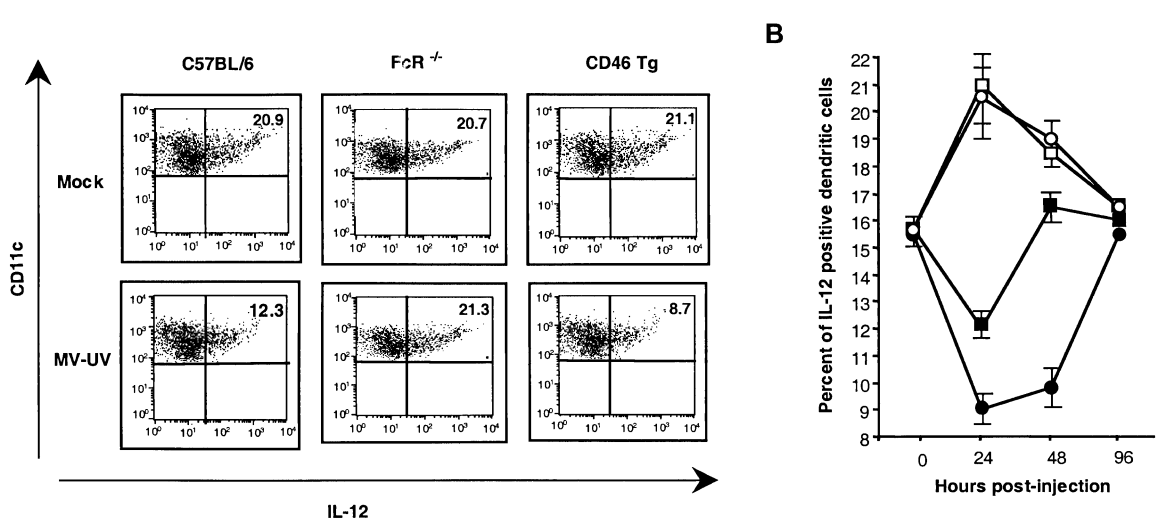

Figure 6. Effect of MV-UV on the Intracellular Production of IL-12 by DC from Draining Lymph Nodes

Groups of three C57BL/6 (squares), CD46 transgenic (circles), or FcR$^{-/-}$ mice were injected with either the mock (open symbols), or 5 $\times$ 10^6 pfu of UV-inactivated MV (solid symbols) and sensitized with DNFB 6 hr later. Draining lymph nodes were pooled at indicated time points after MV-UV/mock injections. Enriched DC were incubated for 4 hr with monensin and subsequently stained for the surface expression of MHC class II and CD11c, followed by cell permeabilization and anti-IL-12-PE intracellular staining.

(A) Representative profile obtained 24 hr after MV-UV/mock injection. MHC class II$^+$ cells were analyzed for the percentage of CD11c$^+$ IL-12$^+$ double-positive cells. IL-12 staining was completely blocked if prior incubation with 0.5 μg of recombinant IL-12 was performed.

(B) Kinetics of intracellular IL-12 production. Results are expressed as the percentage of IL-12$^+$ cells within the MHC class II$^+$ CD11c$^+$ population at different time intervals after mock or MV-UV injection, as mean $\pm$ SD for two experiments.

12 in lymph nodes (Sousa et al., 1997) and the secretion of IL-12 is associated with hypersensitivity responses (Muller et al., 1995), we next examined whether MV proteins could modulate IL-12 production by DC after in vivo hapten sensitization. CD46-transgenic, FcR$^{-/-}$ mice, and nontransgenic controls were either mock or MV-UV injected and sensitized by DNFB 6 hr later. Draining lymph nodes were harvested at regular time intervals, DC were highly enriched, giving the similar yields from each group, and analyzed for IL-12 production by intracellular staining and flow cytometry (Figure 6). In contrast to FcR$^{-/-}$ mice, where injection of MV-UV did not have any effect, in the other two types of mice the percentage of MHC class II$^+$ CD11c$^+$ IL-12$^+$ DC decreased up to 2-fold during the first 24 hr period, being more reduced in CD46-transgenic (8.7%) than in wild-type mice (12.3%) (Figure 6A). Notably, this reduction persisted in CD46 transgenic mice after 48 hr (Figure 6B). Further, no differences were observed in production of TNF-α or IL-10 by the same cell populations 24 hr after the treatment (data not shown). These data demonstrated that injection of MV-UV results in fewer IL-12 producing lymph node DC. The effect was much more pronounced and of a longer duration in CD46-transgenic than in wild-type mice, suggesting a specific role for CD46. Finally, MV-UV did not affect the production of IL-12 in FcR-deficient mice, indicating the important role of FcR in the regulation this DC function.

Immunosuppressive Effect of MV Proteins Requires Expression of FcR on Dendritic Cells

To further analyze mechanism of MV-induced suppression, we purified DC that accumulated in lymph nodes of DNFB-sensitized mice and transferred equal numbers of DC subcutaneously into immunocompetent recipients, which received 6 hr before the transfer, single i.p. injection of either mock or MV-UV. Four days after the transfer, the recipients were challenged with DNFB (Figure 7). As described previously with FITC-primed DC (Kripke et al., 1990), transferred DNFB-primed DC induce a significant CHS response in recipients (Figure 7A). However, when recipients were treated with MV-UV, the efficiency of transferred primed DC to induce CHS response was reduced by half (Figure 7A). In contrast to wild-type DC, the transfer of immunity by DC derived from sensitized FcR-deficient mice into the wild-type was not influenced by MV-UV (Figure 7B), confirming the role of FcR in the immunosuppressive effect of MV and suggesting primed DC as a central target of MV proteins. These results correlate with the absence of immunosuppressive effect of MV-proteins in FcR-deficient mice (Figure 2). Furthermore, when wild-type DC were adoptively transferred from sensitized mice into FcR$^{-/-}$ mice, inhibitory effect of MV-UV was established again (Figure 7C), suggesting that FcR-deficient environment in recipients is not a limiting factor, and emphasizing that FcR expression on DC is critical for the MV-induced immunosuppression. Finally, transfer of DC from CD46 transgenic mice into MV-UV injected wild-type controls resulted with inhibition of hypersensitivity responses (Figure 7D). However, transfer of DC from wild-type into MV-UV-injected CD46 transgenic mice (Figure 7E) entirely abolished hypersensitivity responses, indicating that in addition to DC some other cell type, when expressing CD46, may contribute the MV-induced immunosuppressive effect.

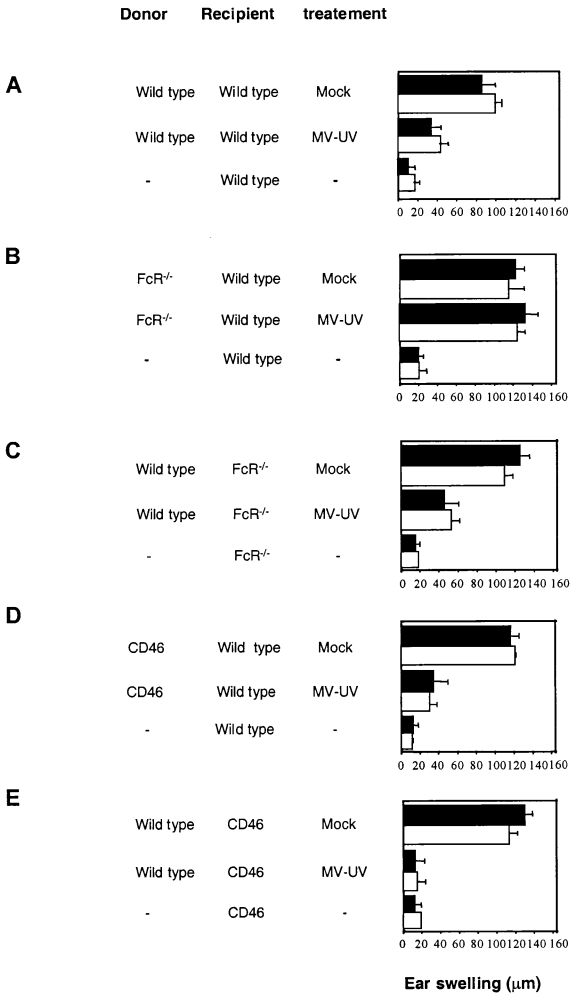

Figure 7. Effect of MV-UV on Adoptively Transferred Immunity by Dendritic Cells

Donor mice, either wild-type (A, C, and E), $FcR^{-/-}$ (B), or CD46 transgenic (D) were DNFB sensitized and DC were purified 18 hr later from regional lymph nodes. Recipient mice, either wild-type (A, B, and D), $FcR^{-/-}$ (C), or CD46 transgenic (E), were injected i.p. with either mock preparation or 5×10^6 pfu of MV-UV and received 6 hr later 5×10^5 DC subcutaneously. Mice were ear-challenged after 4 days with DNFB; results are expressed as mean ear swelling (± SD) at 24 hr (black bars) and 48 hr (white bars) after challenge.

Discussion

In the present study, we demonstrate that MV proteins inhibit two types of T cell–mediated inflammatory responses in mice: CD8-dependent CHS to DNFB and CD4-dependent DTH to KLH. Our results correlate with the disappearance of DTH to tuberculin in children during and after measles infection (Von Pirquet, 1908; Tamashiro et al., 1987), as well as with the important reduction of hypersensitive responses and cellular immunity after measles vaccination (Fireman et al., 1969; Hussey et al., 1996). In addition, suppression of antigen-specific proliferation of T lymphocytes in vitro, observed in our murine model, mimics the lymphoproliferative defect seen in patients with measles. Therefore, the murine hypersensitivity model, described in this study, provides a novel assay to study the mechanism of the measles-induced immunosuppression.

Few peripheral blood mononuclear cells in patients with measles are infected (Esolen et al., 1993), indicating that indirect mechanisms rather than a direct effect of MV replication in lymphoid cells cause suppression. Although more cells may be infected in peripheral lymphoid organs of MV-infected patients, as it has been demonstrated with HIV infection (Frankel et al., 1996), in a recent CD46 transgenic mouse model, peripheral MV infection was detected in less than 0.5% of splenocytes (Oldstone et al., 1999). However, the role of noninfectious virus in the generation of suppression was not addressed in this murine model, and the lack of peripheral MV infection in our CD46 transgenic mice (Horvat et al., 1996; Thorley et al., 1997; Evlashev et al., 2000) prevented us from testing the effect of MV replication. To gain more insight into the mechanism of immunosuppression, this study was focused on the role of MV proteins in the absence of viral replication.

Our results reveal two pathways generating MV-induced suppression of hypersensitivity: one requires an interaction between measles NP and its cellular receptor FcγR, whereas the other implicates virus envelope proteins and the MV-hemagglutinin receptor, CD46. MV nucleocapsid protein binds human and murine Fcγ receptors and inhibits human antibody production in vitro (Ravanel et al., 1997). Here, we show that recombinant NP has a broad immunosuppressive activity and is equally able to inhibit T cell–dependent hypersensitivity in mice. Similar to the effect of MV-UV, inhibition was associated with the suppression of antigen-specific T cell proliferation. Immunosuppressive effect of NP is linked to the last 126 C-terminal amino acids, located in the external part of measles nucleaocapsid, and was completely absent in $FcR^{-/-}$ mice, demonstrating the significance of the NPc-FcR interaction. Liberation of free NP could be expected after an injection of measles virions into mice, and immunosuppressive effect of MV supernatant in nontransgenic mice is most probably composed of scored effects of free NP and released virion-associated NP or its NPc fragment. These findings are important to understand the pathogenesis of MV-induced suppression. During measles infection, the first and most abundant antibodies produced in children are those specific for NP (Graves et al., 1984). Anti-NP antibodies are also detected after vaccination with live MV vaccine (Norrby et al., 1975), indicating a release of MV-nucleocapsid both during infection and after vaccination and its availability to induce suppression. This NP-FcγR interaction may provide a common immunosuppressive pathway for MV strains that use either CD46 or recently identified MV receptor SLAM (CDw152) (Tatsuo et al., 2000). Indeed, both vaccinal and the wild-type strains of MV inhibited hypersensitivity reactions in our study. Efficient immunization against measles is an important public health issue; it is critical for eradication of the disease, but requires additional improvements. Trials using a high-titered attenuated MV vaccine were stopped because of unacceptable morbidity and mortality (Garenne et al., 1991), and its pathogenesis remains to be understood. Further identification of specific NP sequences responsible for the binding of FcR may allow

the development of the new generation of MV vaccines, eventually deprived of immunosuppressive activity.

MV envelope proteins have been suggested to play a role in limiting the capacity of mouse B cells to produce MV-neutralizing antibodies (Fehr et al., 1998). In addition, the coexpression of MV-glycoproteins H and F on UV-irradiated infected cells was reported to lead to a contact-dependent inhibition of mitogen-induced proliferation by noninfected lymphoid cells and cell lines, regardless of CD46 expression (Schlender et al., 1996; Niewiesk et al., 1997). In contrast to these results, a soluble but unidentified factor secreted by MV-infected lymphocytes, suppressing antigen-driven proliferation of uninfected T cells, has been proposed (Sun et al., 1998). Although our data do not exclude the existence of an MV-induced soluble inhibitory factor, they show the role of envelope proteins H and F in the inhibition of hypersensitivity but only in the presence of CD46. This requirement may define the specificity of the MV-envelope proteins in the inhibition of inflammatory responses, which may be absent in the in vitro systems used by others. Whether in these models the recently identified MV receptor SLAM is implicated in the generation of the immunosuppression needs to be analyzed.

Our results demonstrate a significant decrease in dendritic cell IL-12 production from draining lymph nodes of mice hapten-sensitized after i.p. injection of MV-UV, providing in vivo evidence of the systemic modulation of IL-12 production by MV proteins. Consistently with our data, infectious MV and MV-UV inhibit in vitro IL-12 production by activated human dendritic cells (Fugier-Vivier et al., 1997) and LPS-stimulated macrophages (Karp et al., 1996). Both CD46-dependent (Karp et al., 1996) and FcγR-dependent (Sutterwala et al., 1997) mechanism of the inhibition of IL-12 production was demonstrated in human monocytes and murine macrophages, respectively. A reduction in IL-12, demonstrated in our study, was evident in both CD46-transgenic and wild-type mice but absent in FcR-deficient mice, suggesting a role for measles NP-FcR interaction. However, it was more profound and lasted longer in the CD46 transgenic mice, indicating that MV-H interacting with CD46 may cause a greater decrease in IL-12 production and consequent defect in lymphocyte priming, leading to the complete abolition of hypersensitivity. These results suggest existence of different molecular cascades implicated in the regulation of IL-12 production, induced by either FcR or CD46. Furthermore, IFN α/β receptor-deficient mice (Muller et al., 1994) were immunosuppressed at the same level as wild-type mice, when injected with MV-UV (data not shown), indicating that IFN α/β system is not required for FcR-mediated immunosuppression.

Dendritic cells play a critical role in both induction and elicitiation of hypersensitivity reactions and present the cell population that is the most likely to be at the site of virus entry and the early phase of infection. Our results strongly suggest that DC are the major target of MV proteins, related to the profound defect in lymphocyte priming. Consistent with these results, function of human DC and their capacity to stimulate T cells was shown to be impaired after MV infection in vitro (Fugier-Vivier et al., 1997; Grosjean et al., 1997; Schnorr et al., 1997) and at lower extent after the contact with the MV-UV (Fugier-Vivier et al., 1997). Our data further suggested the important role of FcR in MV-induced modulation of DC-mediated initiation of inflammatory reaction. Abnormal maturation and differentiation of activated DC has been recently demonstrated after in vitro infection of human DC (Servet-Delprat et al., 2000). Whether the interaction of MV nucleoprotein with FcR could induce a similar effect in vivo, or affect migration of DC, remains to be determined. Finally, our adoptive transfer experiments using CD46 transgenic mice as recipients suggest the existence of some CD46-expressing cell population, which contributes, in addition to DC, to the induction of immunosuppression. Whether T lymphocytes may be implicated in this effect, as it has recently been demonstrated that stimulation of CD46 could modulate human lymphocyte function (Astier et al., 2000), remains to be analyzed. Altogether, these results suggest a more integral mechanism of MV-induced immunosuppression. Cytopathic viral replication during MV infection (Bellini et al., 1994) or after vaccination could liberate MV proteins or their biologically active fragments, which may than interact with DC via Fc receptors, leading to the altered DC function and reduced IL-12 secretion. Additional viral replication in DC and potential involvement of some other cell populations, expressing CD46, may contribute to this effect. Impaired DC function is responsible for the defective T cell priming, reduces their capacity to respond to foreign antigens and initiate the inflammatory response, which finally results in transient but profound immunosupression.

The potential of MV proteins to modulate DC function and affect both priming and the effector phase of hypersensitivity suggests their possible therapeutic applications, opening new avenues for clinical intervention. Indeed, a marked improvement in symptoms of patients with atopic dermatitis during natural measles has often been observed (Boner et al., 1985; Lin, 1986), and before the development of pharmacological corticosteroids, measles was exploited therapeutically in the treatment of nephrotic syndrome (Blumberg and Cassady, 1947; Hutchins and Janeway, 1947) in spite of risks linked to the infection. Further study of the immunoregulatory role(s) of purified MV proteins and their biologically active fragments, along with toxicology analyses, may open new possibilities of their specific application in immunomodulatory protocols aimed to treat T cell–mediated inflammatory diseases.

Experimental Procedures

Mice

C57BL/6 and BALB/c mice were purchased from IFFA CREDO (L'Arbresle, France). Transgenic mice ubiquitously expressing CD46 (Thorley et al., 1997) were crossed onto the C57BL/6 background. Gene- targeted mice lacking FcR (FcγRII$^{-/-}$ $\times$ FcR$\gamma^{-/-}$, model 585-MM) were obtained from Taconic (USA), and used either as homozygous or heterozygous, after one backcross in C57BL/6 background. Mice deficient for IFN α/β receptor (A129 line) (Muller et al., 1994) were generously provided by R.M. Zinkernagel. CD46 transgenic, FcR$^{-/-}$, and 129/Sv mice were bred in animal house of ENS de Lyon, France.

Virus

Measles virus Edmonston strain was obtained from ATCC (VR-24) and wild-type MV strain, MA93F, was provided by Dr. F. Wild (Lyon,

France). Recombinant MV in which H and F glycoproteins were replaced by G protein of vesicular stomatitis virus (MGV) (Spielhofer et al., 1998), the molecularly cloned Edmonston strain (EDtag) (Radecke et al., 1995), respiratory syncytial virus (RSV) (group A, Long strain), and vesicular stomatitis virus (VSV, Indiana strain) were used for comparative studies. All viruses were propagated on Vero fibroblasts and harvested from infected cells when a strong cytopathic effect developed. Mock preparation contained virus-free supernatant from uninfected Vero cells prepared under the same conditions. In some experiments, MV was additionally purified on a saccharose gradient and gave results identical to nonpurified MV. All viruses were inactivated by 30 min exposure at 4°C to 254 nm UV irradiation and inactivation was confirmed by plaque assay. All virus stocks were free of mycoplasma.

Production of Recombinant and Viral NP and NPc

Recombinant MV nucleoprotein (525 aa) was produced and purified from recombinant baculovirus AcNPVNP, and viral NP was purified from Vero cells infected with MV, as described previously (Ravanel et al., 1997).

DNA corresponding to 126 aa of C-terminal end of NP was cloned into QIAexpress vector pQE-32 (Qiagen), containing 6×Hist epitope tag and then inserted into the baculovirus genome using the pFastBac1 vector and Bac-to-Bac Baculovirus Expression System (Life Technologies), according to the manufacturer's instructions. Insect SF9 cells were transfected with bacmid DNA containing NPc, and recombinant virus Bac-NPc was recovered from the supernatant. Confluent monolayers of High Five insect cells (Invitrogen) were infected with Bac-NPc virus at a multiplicity of 1 PFU/cell. Infected cells were harvested 3 days postinfection, cell pellets were lysed, and NPc was purified according to the Qiagen protocol for high-level expression and purification of 6×His-tagged proteins (The QIAexpressionist).

Assay for Contact Hypersensitivity to DNFB

CHS to DNFB was determined as described (Kehren et al., 1999). In brief, DNFB was diluted in acetone: olive oil (4:1) before use, and 25 μl of 0.5% DNFB solution was applied to a shaved ventral skin (sensitization phase). After 5 days, mice received 10 μl of a nonirritant concentration of DNFB applied on both sides of the left ear and the solvent alone on the right ear (effector phase). Ear thickness was monitored before challenge and every day after challenge for 4 to 6 days, by a third experimenter blinded to sample identity. The ear swelling was calculated as {(T-To) left ear} - {(T-To) right ear}, where T and To are ear thickness after and before challenge, respectively.

Assay for Delayed-Type Hypersensitivity to KLH

DTH response to KLH was measured by a conventional footpad swelling assay. Mice were sensitized by the subcutaneous injection of 200 μl of 300 μg of KLH emulsified with complete Freund's adjuvant (CFA) (1:1, v/v). After 7 days, mice were challenged by the subcutaneous injection in the left hind footpad of 150 μg of KLH diluted in PBS. The right footpad was injected with PBS alone. Footpad thickness was measured before challenge and 24 and 48 hr after challenge by a third experimenter blinded to sample identity. KLH-specific DTH was calculated using the same formula as for CHS.

ELISA

Measles NP content in viral preparations was measured by capture ELISA. In brief, 96-well ELISA plates were coated with anti-NP 33.4 mAb (Libeau et al., 1994), plates were blocked and incubated overnight at 4°C with UV-treated MV supernatants. 1 μg/ml of biotynylated anti-NP Cl.120 antibody (Giraudon and Wild, 1981) was added, and plates were incubated with ExtrAvidin-peroxydase conjugate (1/1000, Sigma, St Louis, MO). Substrate ABTS (Sigma) was added and absorbance at 410 nm was determined after 45 min. A standard curve was established with serial dilutions of purified viral NP.

Preparation of Dendritic Cells

Dendritic cells were purified from lymph nodes as described before (Gunn et al., 1999). In brief, inguinal, axillary, and brachial lymph nodes were pooled from three to four mice. Single cell suspensions were prepared in RPMI 1640 medium (7.5% FCS) and 5×10^6 cells/ml were layered on 2 ml of metrizamide (grade II, Sigma, 14.5 g/100 ml of medium) and centrifuged for 10 min at 600g. Cells at the interface were collected, washed once, and analyzed. Purified cells contained typically 75%–90% DC ($CD11c^+$ $B7\text{-}1^+$ $33D1^+$ $MHC\text{-}II^+$). In some experiments, 90% pure DC (5×10^5) were adoptively transferred subcutaneusly into mice injected 6 hr previously with either mock or MV-UV.

Flow Cytometry

For the detection of NP and NPc binding, 5×10^5 cells were incubated for 1 hr at 4°C with either purified NP (6 μg) or NPc (30 μg) in 100 μl of PBS (1% BSA and 0.1% NaN3). Cells were then washed and incubated with anti-NP biotinylated mAb Cl.25, followed by streptavidin conjugated to FITC staining and analyzed.

For the detection of intracellular cytokines, cell suspensions containing enriched DC (10^6 cells/ml) were incubated with monensin (2 μM GolgiStop) at 37°C in 7% CO_2 for 4 hr, to block cytokine secretion. FcγR was blocked with rat mAb 2.4G2, and cells were stained for surface markers using anti-MHC IA-b,d-FITC (Pharmingen) and anti-N418-biotin (anti-CD11c) followed by streptavidin tricolor (Catlag). Intracellular cytokines were stained using the Cytofix/Cytoperm kit (Pharmingen) according to the manufacturer's instructions and the following antibodies: C15.6-PE (anti-IL-12p40/p70), MP6-XT22-PE (anti-TNF-α), JES5-16E3-PE (anti-IL-10), and isotype control (PE-R3-34 immunoglobulin). In some experiments, the preincubation with 0.5 μg of recombinant IL-12 (R&D Systems) was performed before staining with C15.6-PE antibody. All flow cytometry analyses were carried out on a FACScan (Becton Dickinson).

T Cell Proliferation

Inguinal, brachial, and axillary lymph nodes cells were collected 4 days after DNFB sensitizing. $CD8^+$ T lymphocytes were purified by negative selection using magnetic beads coupled with anti-rat IgG (H&L) as described (Horvat et al., 1996); obtained cell suspensions contained more than 95% $CD8^+$ viable cells. The antigen-presenting cell population was obtained from splenocytes of unsensitized mice and irradiated at 1500 rad. APC were left untreated or pulsed with either DNBS or 2,4,6-trinitrofluorobenzene-sulfonic acid (TNBS) as described (Kehren et al., 1999). In vivo DNFB-primed $CD8^+$ T cells (5×10^5/well) were cocultured in 96-well plates with 10^6 APC, which were either DNBS or TNBS-pulsed or left untreated, in RPMI 1640 medium supplemented with 10% FCS, 10 mM HEPES, 2 mM glutamin, 5×10^{-5}M 2β-ME, and 50 μg/ml of gentamicin for 3 days at 37°C, 7% CO_2. Cell proliferation was assessed on day 3 by ^3H thymidine incorporation for 16 hr. Results were expressed as proliferation indices: (cpm of T cells cultured with hapten-pulsed spleen cells) ÷ (cpm of T cells cultured with untreated spleen cells).

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