Impact of measles virus dendritic-cell infection on Th-cell polarization in vitro

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Interference of measles virus (MV) with dendritic-cell (DC) functions and deregulation of T-cell differentiation have been proposed to be central to the profound suppression of immune responses to secondary infections up to several weeks after the acute disease. To address the impact of MV infection on the ability of DCs to promote Th-cell differentiation, an in vitro system was used where uninfected, tumour necrosis factor alpha/interleukin (IL) 1/β-primed DCs were co-cultured with CD45RO+ T cells in the presence of conditioned media from MV-infected DCs primed under neutral or DC-polarizing conditions. It was found that supernatants of DCs infected with an MV vaccine strain strongly promoted Th1 differentiation, whereas those obtained from wild-type MV-infected DCs generated a mixed Th1/Th0 response, irrespective of the conditions used for DC priming. Th-cell commitment in this system did not correlate with the production of IL12 p70, IL18 or IL23. Thus, a combination of these or other, as yet undefined, soluble factors is produced upon MV infection of DCs that strongly promotes Th1/Th0 differentiation.

INTRODUCTION

Despite the availability of an efficient live vaccine, measles virus (MV) infections are still associated with high morbidity and mortality rates worldwide. A virus-specific immune response established in the course of acute measles leads to efficient virus clearance and long-lasting immunity. Paradoxically, at the same time, immune reactions to secondary infections are profoundly, yet transiently, suppressed. The resulting high susceptibility to opportunistic infections (Griffin, 1995) accounts almost exclusively for infant death associated with measles. MV-induced immunosuppression is characterized by marked lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment induced immunosuppression is characterized by marked lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & Biberfeld, 1983) and a general failure of lymphopenia that mainly affects the T-cell compartment (Arneborn & 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infectious MV by infected DCs to T cells is, however, inefficient (Fugier-Vivier et al., 1997). MV infection of immature DCs causes rapid phenotypic maturation, as indicated by the augmentation of major histocompatibility complex (MHC) class I and II and CD40 expression and the induction of CD86, CD80 and CD83. Type I interferon (IFN) was found to account, at least partially, for this phenomenon (Dubois et al., 2001; Klagge et al., 2000; Servet-Delprat et al., 2000b). Despite their activated phenotype, MV-infected DCs fail to stimulate allocogeneic T-cell proliferation in vitro in a mixed leukocyte reaction; for this, negative signalling by the viral glycoproteins expressed on their surface was found to be essential (Dubois et al., 2001; Klagge et al., 2000). There is also evidence that apoptosis of both DCs and T cells occurs in infected cultures and that this is brought about by TRAIL [tumour necrosis factor (TNF)-related apoptosis-inducing ligand] or Fas ligation, respectively (Servet-Delprat et al., 2000a; Vidalain et al., 2001).

Whilst cytokine release from infected DCs may not affect T-cell proliferation directly, it would be expected to have a significant impact on the quality of the ensuing T-cell response (referred to here as Th-cell polarization). In this regard, the regulation of interleukin 12 (IL12) induction by MV has been a major focus of research, albeit with variable results. In monocyte cultures, ligation of CD46 by antibodies, C3b/C4b complement components or MV strains that interact firmly with this molecule was found to interfere with IL12 induction (Karp et al., 1996). Similar observations with regard to IL12 induction by lipopolysaccharide (LPS) or CD40 ligation were made in MV-infected DC cultures, although the underlying mechanism has not been resolved (Fugier-Vivier et al., 1997). Moreover, stimulated IL12 production by peripheral blood cells of measles patients was found to be suppressed for prolonged periods (Atabani et al., 2001). Conversely, induction of IL12 in DCs early after infection was found by other studies to be unaffected, or even enhanced (Dubois et al., 2001; Schnorr et al., 1997; Servet-Delprat et al., 2000b). None of these studies, however, directly addressed the impact of MV-infected DCs on T-cell commitment.

We provide evidence that IL12 release from CD40L-stimulated DCs is not suppressed early in infection. By using a modified co-culture system that was initially described by de Jong et al. (2002), we show that uninfected, TNF-α/IL1β-matured DCs potently promote differentiation of IFN-γ-producing T cells in the presence of conditioned media from DCs infected with the Edmonston (ED) vaccine strain of MV, indicating a type 1 response or a mixed type 0 response. This was also observed when the DCs used for ED infection were primed under conditions that normally favour their ability to drive a type 2 response. Supernatants of DCs infected by wild-type MV, such as WTP or Bilthoven, did not show this strong Th1-inducing activity, but favoured a general Th0 cytokine profile. Supernatants of MV-infected DCs did not promote the differentiation of Th2 cells under any conditions. However, T-cell differentiation did not correlate strictly with the induction of IL12, IL18 or IL23 in the DC cultures.

METHODS

Viruses and cells. Vero cells were cultured in minimal essential medium/5% fetal calf serum (FCS), BJAB cells (human B- lymphoblastoid cells) in RPMI 1640/10% FCS and B95a cells (marmoset B-lymphoblastoid cells) in Dulbecco’s modified Eagle’s medium/5% FCS. In addition to penicillin/streptomycin, all media were supplemented with ciprofloxacin (final concentration, 10 μg ml−1; Bayer). The vaccine strain Edmonston was grown and titrated on Vero cells and the wild-type strains WTP and Bilthoven (Bil) were grown on BJAB cells; TCID50 was determined on B95a cells. Cell cultures and virus preparations were tested routinely for mycoplasma contaminations by PCR (Myco-PCR detection kit; Minerva Biolabs) or by using an indicator cell line (Bieback et al., 2002).

Generation of immature DCs and their induction of maturation. Whole blood samples were obtained by leukapheresis from healthy volunteers (Department of Transfusion Medicine, Würzburg University, Germany). Monocytes were isolated by Ficoll density centrifugation followed by Percoll density centrifugation (Amersham Biosciences). Immature DCs were generated by culturing monocytes for 6 days in RPMI 1640/10% FCS containing streptomycin/penicillin supplemented with granulocyte–macrophage colony-stimulating factor (GM-CSF) (500 U ml−1; Novartis Pharma) and IL4 (250 U ml−1; Strathmann Biotec). Immature DCs were harvested and their maturation was induced by adding the following compounds for 2 days to the cultures alone or in combinations, as detailed in the text: TNF-α (5000 U ml−1; Strathmann Biotec), IL1/β (500 U ml−1; Strathmann Biotec), 1 μg cholera toxin (CTx) ml−1 (Sigma-Aldrich), 10−5 M histamine (Sigma-Aldrich). Alternatively, immature DCs were co-cultured for 2 days with CD40L-expressing L cells at a 1:1 cell ratio (a kind gift from Dr A. Schimpl, Würzburg University, Germany) to induce DC maturation.

Infection of DCs and generation of conditioned supernatants of DC cultures. DCs were infected with the different MV strains at an m.o.i. of 0.05 p.f.u. and cultured in 24-well plates at a density of 105 cells ml−1 under neutral conditions (GM-CSF/IL4 or GM-CSF/ IL4/IL1β/TNF-α); under Th1 conditions (GM-CSF/IL4/IL1β/TNF-α plus CD40L-expressing L cells); or under Th2 conditions (GM-CSF/IL4/IL1β/TNF-α plus CTx or histamine). After 2 days, the cultures were harvested and the cell-free supernatants collected and stored at −80 °C before use.

Cytokine polarization of naïve CD4+ T cells. naïve T cells were purified from CD2+ lymphocytes obtained after rosetting whole blood samples with sheep red blood cells (Virion) by magnetic depletion using a mixture of CD8, CD56 and CD45RO MicroBeads (Miltenyi Biotec). Cytokine polarization of the naïve T cells was accomplished by using a modified method described by de Jong et al. (2002). DCs matured under neutral conditions were co-cultured with naïve T cells at a ratio of 1:10 in 24-well cell-culture plates (Greiner) in the presence of staphylococcal enterotoxin B (SEB) (100 pg ml−1; Sigma-Aldrich). To avoid infection of DCs and T cells, conditioned supernatants of infected DC cultures were separated from the co-cultures by transwell filter inserts (pore size, 0.02 μm; Nunc). At day 5 of co-culture, IL2 (10 U ml−1; Strathmann Biotec) was added. The maturation and infection status of the T cells was controlled routinely [using a CD45RO-Phar (Pharmingen) or N-specific antibody, F227, generated in our laboratory] by using flow cytometry. At day 14, the expanded T-cell populations showed a homogeneous CD45RO expression and were
restimulated with phorbol myristate acetate (PMA) (50 ng ml\(^{-1}\); Sigma-Aldrich) and ionomycin (1 \(\mu\)g ml\(^{-1}\); Sigma-Aldrich) for 6 h in the presence of Brefeldin A (10 \(\mu\)g ml\(^{-1}\); Sigma-Aldrich) during the last 5 h of stimulation. After formaldehyde fixation and subsequent saponin permeabilization, cells were stained with fluorescein isothiocyanate (FITC)-conjugated anti-IFN-\(\gamma\) and phycoerythrin (PE)-conjugated anti-IL4 PE antibodies (BD Biosciences). Cells were analysed on a FACS Calibur (BD Biosciences) using CellQuest Pro Software (BD Biosciences). Student’s \(t\)-test was performed for statistical analysis.

**Cytokine measurements.** IL12 p70, IL12 p40 and IL10 concentrations were determined by using specific ELISA sets, according to the manufacturer’s instructions (R&D Systems).

**RNA isolation, cDNA preparation and PCR analysis.** Total RNA was purified from DCs 2 days after mock or MV infection by using an RNeasy mini kit (Qiagen). Complementary DNA was generated by using a first-strand cDNA synthesis kit for RT-PCR (MBI Fermentas). Primer sequences (each indicated in the 5’–3’ orientation) used were: IL18 (forward: GCTTGAATCTAAATATTACG; reverse: GAAGATTCAAATTGCACTTAT) yielding a 342 bp product; IL23 p19 (forward: AGCAGCTCAAGGATGCCACTG; reverse: CCCCCCTCTTCCTCCACTA) yielding a 100 bp product (Wesa & Galy, 2002); \(\beta\)-actin (forward: TGACCAGATCATGTTTGAGA; reverse: ACTCCATGCCCAGGAAGGA) yielding a 456 bp product. PCR conditions were as follows: IL18, 5 min at 94 °C, 30 cycles (30 s at 96 °C, 1 min at 57 °C, 30 s at 72 °C) and 10 min at 72 °C; IL23 p19, 5 min at 94 °C, 35 cycles (1 min at 95 °C, 2 min at 56 °C, 1 min at 70 °C) and 10 min at 72 °C; \(\beta\)-actin, 5 min at 94 °C, 30 cycles (1 min at 95 °C, 1 min at 60 °C, 1 min at 72 °C) and 10 min at 72 °C. PCR products were separated on 1.5% (for IL18) or 2% agarose gels containing ethidium bromide. A 100 bp DNA ladder (MBI Fermentas) served as size marker.

**RESULTS**

**Priming conditions of human monocyte-derived DCs induce differential Th-cell polarization**

In order to investigate Th-cell polarization by MV-infected DCs, we first needed to modify the experimental system described by de Jong \textit{et al.} (2002), in which T-cell differentiation was analysed 2 weeks after co-culture of activated DCs with naïve CD4\(^+\) T cells. Direct co-culture of MV-infected DCs with uninfected T cells is not applicable, as MV-infected DCs expressing the MV glycoprotein complex signal negatively to T cells via a surface contact and thereby prevent T-cell expansion (Klagge \textit{et al.}, 2000; Dubois \textit{et al.}, 2001). Moreover, despite inefficient transmission of infectious virus by DCs, up to 50% infected T cells were detectable after 14 days co-culture of infected DCs with naïve T cells, due to virus spread within the T-cell population (not shown). Therefore, monocyte-derived DCs were kept in GM-CSF/IL4 or modified by addition of either TNF-\(\alpha\)/IL1\(\beta\) (referred to as ‘neutral conditions’), histamine or CTx (to allow for subsequent Th2 commitment) or co-cultured with CD40L-transfected fibroblasts (to allow for subsequent Th1 commitment) for a further 2 days culture. Treatment with either compound in combination with TNF-\(\alpha\)/IL1\(\beta\) induced phenotypic maturation of DCs. As reported, high amounts of IL12

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**Fig. 1.** Induction of cytokines in MV-infected DC cultures. Immature DCs were infected with MV or mock preparations as indicated and cultured under various stimulatory conditions. After 2 days, the supernatants were collected and cytokine concentrations were determined by ELISA. (a) IL12 p40; (b) IL12 p70; (c) IL10. Results are representative of five experiments. (d) Total RNA isolated from uninfected or infected DCs was subjected to RT-PCR analysis for IL18 and IL23 p19 gene products. PCR specific for \(\beta\)-actin serves as a control. Co, Control PCR in the absence of template. One of three independent experiments is shown.
p70 were produced only from DCs that were activated by CD40 ligation, whereas this cytokine was barely released from DCs kept under neutral conditions or primed by CTx or histamine (Fig. 1b). Autologous naïve CD45RO⁺ T cells were then co-cultured with uninfected SEB-pulsed DCs (activated by TNF-α/IL1β) in the presence of conditioned media that were obtained from the differentially activated DC cultures. These supernatants were separated from the DC/T-cell co-cultures by a filter that allows for transfer of soluble mediators, but not MV. After 2 weeks co-culture, the purified naïve CD4⁺ T cells expanded 50- to 200-fold and acquired a CD45RO⁺ memory phenotype (Fig. 2a). Intracellular stainings for IFN-γ and IL4 revealed that under neutral conditions (supernatants of immature or TNF-α/IL1β-activated DCs), the T cells showed no clear Th polarization. Supernatants of CD40L-activated DCs led to the differentiation of Th1 cells, whereas DC stimulation with CTx or histamine resulted in supernatants favouring the development of Th2 cells (Table 1). In addition, T cells producing both IL4 and IFN-γ were also detected (Table 1, third column).

### Impact of MV infection of DCs on Th-cell polarization

MV infection of DCs gave rise to extended syncytia formation after 2 days. Syncytia formation occurred more quickly and was more pronounced with the MV wild-type

### Table 1. IFN-γ and IL4 production by CD4⁺ T cells induced by co-culture with DCs in the presence of conditioned cell supernatants of uninfected, differentially matured DCs. Percentages are calculated as means ± SD from five independent experiments

<table>
<thead>
<tr>
<th>Stimulation</th>
<th>IFN-γ (%: ± SD)</th>
<th>IL4 (%: ± SD)</th>
<th>IL4/IFN-γ (%: ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GM-CSF/IL4</td>
<td>12:72 ± 8:9</td>
<td>9:02 ± 8:3</td>
<td>2:51 ± 2:5</td>
</tr>
<tr>
<td>TNF-α/IL1β</td>
<td>12:41 ± 8:0</td>
<td>2:86 ± 5:5</td>
<td>1:40 ± 0:9</td>
</tr>
<tr>
<td>CD40L</td>
<td>53:47 ± 8:8</td>
<td>2:61 ± 1:1</td>
<td>3:66 ± 1:3</td>
</tr>
<tr>
<td>CTx</td>
<td>2:73 ± 2:9</td>
<td>10:12 ± 1:4</td>
<td>1:49 ± 1:4</td>
</tr>
<tr>
<td>Hist</td>
<td>3:06 ± 2:3</td>
<td>13:30 ± 4:0</td>
<td>1:23 ± 1:1</td>
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strain WTF than with the MV vaccine strain ED, irrespective of the different DC-priming conditions (Fig. 3). Later, MV-infected DCs disintegrated rapidly (not shown). To analyse the impact of MV-infected DCs on Th-cell polarization, the supernatants of infected and uninfected DC cultures grown under various conditions were harvested 2 days post-infection (p.i.) and used in our modified DC/T-cell co-culture assay. After 14 days culture, T cells were viable, CD45RO⁺ and did not stain for the MV H protein, indicating that infection was prevented efficiently (not shown). Following activation by PMA/ionomycin, T cells were analysed for IFN-γ and IL4 cytokine production by intracellular staining. When exposed to conditioned media from mock-infected DCs, T cells acquired a predominantly Th0 phenotype with supernatants of DCs primed by GM-CSF/IL4; a Th2 phenotype (predominantly IL4-producing cells) by CTx or histamine-primed DC supernatants; and a Th1 phenotype (predominantly IFN-γ-producing cells) by supernatants from CD40L-activated DCs (Figs 2b–d and 4a). T cells expressing both IFN-γ and IL4 were barely induced by supernatants of mock-treated DCs under all priming conditions used (Fig. 4b). Surprisingly, conditioned media from ED-infected (and, albeit less efficient, wild-type MV-infected) DCs, irrespective of DC priming, shifted the response to a Th1 type with a high percentage of IFN-γ-producing T cells (Figs 2c and d and 4a). In addition, an increased proportion of IFN-γ⁺/IL4⁺ T cells was seen (Fig. 4b). Supernatants of CD40 ligand-activated DCs infected with wild-type MV induced a strong Th1 response (Fig. 4a), whereas those obtained from infected DCs primed with GM-CSF/IL4 alone, CTx and histamine led to only a modest increase in the proportion of IFN-γ-producing T cells (Th0 response) (Fig. 4a). The amount of IFN-γ⁺/IL4⁺ T cells produced under these conditions did not differ significantly from that seen with the mock supernatants (Fig. 4b). Thus, even under DC-priming conditions favouring a Th2 response, conditioned media from ED-infected DCs are able to favour a highly significant differentiation into IFN-γ-producing T cells, as did those of WTF- or Bil-infected DCs, which were also significantly efficient. As the percentage of IL4-producing T cells did not differ significantly from that seen with the mock control, supernatants of ED-infected DCs trigger a Th1, whereas those of wild-type MV-infected DCs trigger a type 0 response under CTx- and histamine-priming conditions.

### Cytokine production from infected DCs

To address the role of cytokines produced by MV-infected DCs in Th-cell polarization, supernatants were analysed 2 days after MV or mock infection. IL12 p40 was produced...
readily from the differentially primed DCs, irrespective of MV infection (Fig. 1a). In contrast, bioactive IL12 p70 was released from TNF-α/IL1β-primed DCs only after CD40 ligation and, interestingly, MV infection did not interfere with the production of this cytokine (Fig. 1b). Under the same conditions, IL10 was released from mock- and MV-infected DCs (Fig. 1c). As IL18 and IL23 are known to promote Th1 differentiation under certain conditions, we analysed production of these cytokines in DCs that were generated under neutral conditions (GM-CSF/IL4) 2 days after MV infection by RT-PCR. Transcripts specific for IL18 (Fig. 1d, upper panel) were induced readily by infection with both the ED and the two wild-type strains tested, but also by mock treatment, indicating that IL18 alone is not likely to contribute to Th-cell commitment in our system. In contrast, detectable levels of the p19 subunit of IL23 were induced slightly by mock treatment and moderately by the ED strain, but not by the two wild-type strains (Fig. 1d, middle panel). As IL23 p19 is induced slightly in mock, but not wild-type MV-infected DCs (the supernatants of which induce Th1-cell commitment more efficiently than those of mock-infected DCs), this cytokine alone is also unlikely to contribute to Th differentiation by MV.

DISCUSSION

The issue as to whether MV infection or vaccination favours a predominant Th2 response and whether this would be linked to MV-induced immunosuppression is still a matter of debate (Gans et al., 1999; Griffin et al., 1989, 1990; Schnorr et al., 2001; Ward & Griffin, 1993). The interaction of MV with DCs has been suggested to be central to MV-induced immunosuppression, although direct infection of these cells in vivo has not been shown as yet. In vitro, DCs (generated from CD34+ precursor cells or monocytes, or isolated from peripheral blood) and Langerhans cells were found to be readily infectable by MV (Dubois et al., 2001; Fugier-Vivier et al., 1997; Grosjean et al., 1997; Klagge et al., 2000; Ohgimoto et al., 2001; Schnorr et al., 1997; Servet-Delprat et al., 2000b; Steineur et al., 1998). The haemagglutinin protein of MV wild-type strains apparently confers a tropism of these strains for DC infection (Ohgimoto et al., 2001); this is reflected by faster kinetics of syncytia formation, accumulation of MV H protein on the surface of these cells and induction of phenotypic maturation of DCs (Schnorr et al., 1997; Fig. 3). Although CD40 ligation was found to promote MV replication in DCs (Fugier-Vivier et al., 1997), in our system, neither syncytia formation
Among APC effector functions that are potentially modulated by MV, regulation of IL12 p70 production has received major attention. Suppression of LPS- or *Staphylococcus aureus*-induced IL12 p70 production from monocytes was related to MV-mediated ligation of CD46 (Karp *et al*., 1996). This, although not formally proven, suggests that CD46 ligation by attenuated MV strains may interfere with signalling via Toll-like receptor 2 or 4. In DCs isolated from peripheral blood, LPS- or SEB-induced IL12 p70 production was enhanced slightly 2 days after infection with either MV ED or WFT (Schnorr *et al*., 1997) and increased levels of IL12 p40 and IL12 p35 mRNAs were observed early after infection with an attenuated MV strain in the absence of stimulation of DCs (Servet-Delprat *et al*., 2000b). From our experiments, however, there is no indication that MV infection alone induces production of bioactive IL12 p70 or IL12 p40 to greater extents than the mock control (Fig. 1a, b). Whilst CD40L-induced accumulation of IL12 p40 mRNA was found to be reduced 24 h after MV infection in one study (Servet-Delprat *et al*., 2000b), IL12 p70 production from DCs generated from CD34+ precursors was found to be enhanced by the MV ED strain 3 days after CD40 ligation (Dubois *et al*., 2001). Our results indicate that MV infection (with either the ED or the wild-type strain WTF) did not affect the CD40L-induced release of bioactive IL12 p70 from DCs (Fig. 1b). This apparently contrasts with a strong reduction in IL12 p70 release from CD40L-activated DCs infected with an attenuated MV strain (Fugier-Vivier *et al*., 1997). In these studies, the inhibitory effect was, however, most pronounced later than 3 days p.i., when a significant number of DCs had already undergone apoptosis. It thus appears that, at least within the first 2 days after infection, CD40L-induced IL12 p70 is not affected by MV; this may be of crucial importance for the induction of an MV-specific immune response.

Based on *in vitro* experiments with MV-infected DCs, mechanisms that have been proposed to account for immunosuppression include DC or lymphocyte loss by mechanisms that have been proposed to account for immunosuppression include DC or lymphocyte loss by apoptosis (Fugier-Vivier *et al*., 1997; Grosjean *et al*., 1997; Servet-Delprat *et al*., 2000a) or cytotoxic activity of DCs, which was found to be dependent on IL12. It has also been shown that commitment to a type 1 response after infection with *Salmonella typhimurium* occurs in an IL12-independent fashion, whilst IL12 is required for maintenance of the response (John *et al*., 2002).

As bioactive IL12 p70, which promotes differentiation of naïve CD4+ T cells to Th1 cells and thereby induces IFN-γ production, is only released from MV-infected DCs after CD40 ligation (Fig. 1b), type 1 responses, as induced by MV-infected DCs kept under neutral conditions or in the presence of histamine or CTx, have to be generated independently of this cytokine. The ability of viruses to induce Th1 responses in the absence of IL12 has been described previously. Thus, IL12 p40-deficient mice with lymphocytic choriomeningitis virus, vesicular stomatitis virus or mouse hepatitis virus infections efficiently generated a type 1 response (as indicated by production of type 1 cytokines) and mounted a protective immunity (Oxenius *et al*., 1999; Schijns *et al*., 1998). In contrast, IL12 p40-deficient animals were unable to control infections with *Listeria monocytogenes*, indicating that, for some bacterial infections, induction of Th1 responses is crucially dependent on IL12. It has also been shown that commitment to a type 1 response after infection with *Salmonella typhimurium* occurs in an IL12-independent fashion, whilst IL12 is required for maintenance of the response (John *et al*., 2002).

IL10 is known to be induced upon MV infection of DCs, but seems not to be responsible for the inability of infected DCs to promote T-cell expansion (Dubois *et al*., 2001). This cytokine is also unlikely to contribute to T-cell commitment in our cultures, as IL10 was produced only from DCs that were activated by CD40L and the levels released from MV wild type-infected DCs were equivalent to those seen in the mock-infected controls (Fig. 1c).

Type I IFNs can enhance T-cell IFN-γ responses in human cells (O'Shea & Visconti, 2000). Although we found production of type I IFN from ED-infected and, to a lesser extent, from WTF-infected DCs, this cytokine is unlikely to play a major role in our system, as inclusion of type I IFN-neutralizing antibodies did not affect Th-cell polarization (not shown). Furthermore, type I IFN, as present in the conditioned media, was added to co-cultures of CD45RO+ T cells and TNF-α/IL1β-matured DCs, which led to TNF-α/IL1β-mediated DCs.
as described recently, type I IFN, when present during maturation, generates DCs that strongly promote Th1 polarization, this cytokine was found to inhibit differentiation into Th1 effectors cells by mature DCs (Nagai et al., 2003). In this system, the ability of TNF-α/IFN-β-matured DCs to promote Th1 differentiation correlated with enhanced production of IL23 and IL18, with the latter being identified as the major factor (Nagai et al., 2003). In contrast to IL12, IL23 promotes proliferation and IFN-γ secretion preferentially in activated human memory Th cells and has little or no effect on naïve Th cells (Oppmann et al., 2000). As we used CD45RO− T cells for the initial priming, this cytokine alone is unlikely to contribute to Th-cell differentiation in our system. Furthermore, IL23 p19-specific transcripts are not induced upon DC infection with wild-type MV strains, and it is unlikely that this cytokine accounts for Th-cell commitment in our system alone (Fig. 1d). Although the third member of the IL12 family, IL27, can promote expansion and IFN-γ production of stimulated naive, but not memory, Th cells, it only does so synergistically with IL12 (Robinson & O’Garra, 2002). As we have no evidence that the cell commitment in our system is dependent on IL12 p70 production (see above), it appeared unlikely that IL27 would be involved; therefore, production of this cytokine was not analysed. Also, no differential accumulation of IL18-specific transcripts was detected in mock- versus MV-infected DCs. Therefore, IL18 apparently did not contribute to the induction of Th1 or Th0 differentiation that was seen here (Fig. 1d). It appears that (a) novel cytokine(s), not identical to IL12, IL18 or IL23, and potentially also including IFN-γ (which was not specifically addressed in this study), may be produced from MV-infected DCs that strongly promote(s) Th1 or Th0 commitment. The identification of these warrants future experimentation.

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