Vesicular stomatitis virus and pseudorabies virus induce a \textit{vig1/cig5} homologue in mouse dendritic cells via different pathways

Pierre Boudinot,1 Sabine Riffault,1 Samia Salhi,1† Charles Carrat,1 Christine Sedlik,2 Nassira Mahmoudi,1 Bernard Charley1 and Abdenour Benmansour1

1 INRA, Unité de Virologie et Immunologie Moléculaires, 78352 Jouy-en-Josas cedex, France
2 INSERM U520, Institut Curie, section recherche, 12 rue Lhomond, 75005 Paris, France

The homologous genes \textit{vig1} and \textit{cig5} were identified by differential display PCR as virus-induced genes in rainbow trout and humans, respectively. These genes are significantly related to sequences required for the biosynthesis of metal cofactors, but their function remains unknown. In this study, it is shown that the mouse homologue of \textit{vig1/cig5} was induced by vesicular stomatitis virus (VSV) and pseudorabies virus (PrV) in mouse spleen cells. Among a collection of cell lines from dendritic, myeloid, lymphoid or fibroblast lineages, only the dendritic cell line, D2SC1, showed expression of \textit{mvig} after virus infection. This dendritic restriction was confirmed by our finding that \textit{mvig} was also induced by both VSV and PrV in CD11c++ spleen cells, separated by magnetic purification or derived from bone marrow precursor cells. Similar to the fish rhabdovirus viral haemorrhagic septicaemia virus in trout cells, VSV directly induced \textit{mvig} in the dendritic cell line D2SC1, but the PrV-mediated induction required the integrity of the interferon pathway. This result indicates that \textit{mvig} is interferon-inducible like its fish and human homologues. Furthermore, \textit{mvig} was also induced by LPS in bone marrow-derived cells. Thus, \textit{mvig} expression seems to correlate with an activated state of dendritic cells subjected to different pathogen-associated stimuli.

Introduction

Infection with viruses is known to lead to a dramatic shift in the transcriptional activity of host cellular genes. These changes have been shaped by the long co-evolution of the host and the pathogen and reflect the two opposing strategies developed by the virus to subvert the cell metabolism and the host to counter virus infection (Hardwick & Griffin, 1996). Recently, new methodologies were developed for the global analysis of cell transcripts. The transcriptional shift due to viruses has been studied using mRNA differential display (Hsiang et al., 1996; Sorbara et al., 1996; Zhu et al., 1997; Tal-Singer et al., 1998; Boudinot et al., 1999; Zhang et al., 1999), oligonucleotide array technology (Zhu et al., 1998) and DNA microarray hybridization (Geiss et al., 2000). Many genes were proved to be increased or decreased in such systematic studies, some of which potentially play important roles in virus-induced pathogenesis or in antiviral response.

Using differential display PCR, we have described \textit{vig1} as a gene induced during virus incubation of rainbow trout leukocytes with viral haemorrhagic septicaemia virus, a fish rhabdovirus (VHSV; Boudinot et al., 1999). Its human homologue, \textit{cig5}, was similarly identified from primary skin cultures incubated with inactivated human cytomegalovirus (Zhu et al., 1997). \textit{vig1} and \textit{cig5} sequences are very similar, suggesting a conserved function probably related to the host response to virus infection. The rat gene \textit{best5} identified in bone tissue from ovariectomized females (Grewal et al., 2000) and mouse expressed sequence tags from different tissues display high

\begin{flushleft}
\textbf{Author for correspondence:} Abdenour Benmansour.
\texttt{Fax +33 1 34 65 25 91. e-mail abdenour@biotec.jouy.inra.fr}
\end{flushleft}

† \textbf{Present address:} Unité de Biochimie Cellulaire, FRE 2219 CNRS, Université Pierre et Marie Curie, 9 quai Saint Bernard, 75005 Paris, France.

The GenBank accession number of the sequence reported in this paper is AF236064.
levels of sequence similarity with vig1 and cig5. This conservation suggests that these genes, present in all vertebrates, may form a new family of genes implicated in the cell non-specific response. The vig1/cig5 gene family shares the MoaA sequence signature with several prokaryotic and eukaryotic genes. The MoaA motif (Prosite PDOC1009) is present in bacterial, plant and human proteins involved in the synthesis of enzymatic cofactors such as pyrroloquinoline quinone (PQQ3), haem D1 and molybdopterin (Hoff et al., 1995; Reiss et al., 1998). Such cofactors can play different roles. For example, a tetrahydrobiopterin cofactor modulates the activity of the inducible nitric oxide synthase. Accordingly, the vig1/cig5 gene family may be implicated in the synthesis of a cofactor participating in an enzymatic pathway induced in pathological conditions. However, the function of these genes remains to be elucidated.

The mouse appears as a more appropriate model to gain further insight into the function of such genes. To compare with the situation described in man and the rainbow trout, we used a rhabdovirus, vesicular stomatitis virus (VSV), as virus inducers. We first confirmed that both viruses induced the mouse gene homologous to vig1 and cig5 (mvig). We then identified dendritic cells as the main cell population where the up-regulation of the inducible nitric oxide synthase takes place. The vig1/cig5 gene family may represent a new class of primitive mediators active during infection in vertebrates.

Methods

- **Mice.** C57Bl/6 mice (8 weeks old) were bred in the INRA animal facilities at Jouy-en-Josas, France.

- **Virus preparation.** The Bartha strain of PrV was propagated in porcine kidney cells (PK15) in serum-free MEM. Supernatants, collected after 2 days of infection, were clarified and the virus titre was determined on PK15 cells and expressed in TCID$_{50}$/ml (1 x 10$^6$). The Indiana laboratory strain (Orsay) of VSV was grown for 24 h in BSR cells (a clone of BHK-21) in MEM supplemented with 2% FCS. The virus titre was determined by plaque titration and expressed in pfu/ml (typically 1 x 10$^4$). When necessary, VSV was inactivated with β-propiolactone at 1/4000 for 1 h at room temperature and then overnight at 4 °C.

- **Dendritic cell preparation.** Positive selection of spleen dendritic cells was performed by magnetic cell sorting, with the VarioMACS (Miltenyi Biotech), using anti-CD11c (N418)-conjugated magnetic beads (#520-01) and a VS+ column. Positively selected cells were passed over a second selection column (MS+) to increase their purity. Purity of selected and depleted cells was verified by control staining of CD11c-positive cells with an FITC-conjugated antibody specific for hamster IgG(H+L) (Caltag). Rat anti-class II antibody was TIB120 (ATCC no. 3480), and was used with an FITC-conjugated anti-rat kappa light chain (Pharmingen).

Bone marrow-derived dendritic cells were obtained as described by Winzler et al. (1997). Briefly, bone marrow cells were cultured for 2 weeks in Iscove's modified Dulbecco's medium (Sigma) containing 10% heat-inactivated FBS (GIBCO), 100 IU/ml penicillin, 100 µg/ml streptomycin, 2 mM t-glutamine (Sigma), 50 µM 2-mercaptoethanol, in the presence of 30% conditioned medium from GM-CSF-producing NIH/3T3 cells (Regnault et al., 1999; Winzler et al., 1997). Antibodies specific for CD11c (N418), GR-1, CD86/B7-2(GL1) and CD40 (HM40-3) were from Pharmingen, and the anti-class II MHC was Y3P (ATCC no. 5168).

- **Cell stimulation conditions.** Cells were adjusted to 2–5 x 10$^5$/ml and infected with VSV or with PrV diluted 1/100 in culture medium. For each cell type tested, a control with no virus was included. Cells were incubated for 3–5 h at 37 °C. In addition, mock infections (i.e. incubation with supernatants from non-infected PK15 or BSR cells) were performed when relevant. Cycloheximide (CHX; Sigma) was used at 100 µg/ml. Anti-mouse α/β interferon immunoglobulin (neutralizing titre of 5 x 10$^7$/ml against L-cell-derived α/β interferon) was provided by B. Dalton (SmithKline Beecham Pharmaceuticals). Normal sheep immunoglobulin was purchased from the National Veterinary Institute, Uppsala, Sweden. LPS from E. coli 026:B6 (Sigma) was used at 1 µg/ml.

- **mvig cloning and sequencing.** Total RNA isolated from lymph node cells according to the method of Chomczynski was treated with 10 U of Dnase I (Boehringer Mannheim) in 0-1 M sodium acetate, 5 mM MgSO$_4$, pH 5, with 100 µM of ribonuclease inhibitor (RNaseOUT, GibcoBRL) at 1 h at 37 °C. DNase I was eliminated by phenol and chloroform extraction. RNA from each sample (5 µg) was reverse transcribed using 400 U of M-MLV reverse transcriptase with 7 mM of random hexanucleotide primers (pNd), Pharmacia Biotech), 10 mM of each dNTP and 40 U of RNaseOUT. A primer pair derived from the rat sequence (GenBank accession no. Y07704), MV1 forward 5' GCTCGGCTGCTGACCTGTTCC 3' and MV1 reverse 5' AACAAAGCCTGTAGACAGC 3' (amplified product 1157 bp), was used to amplify a fragment encompassing most of the vig ORF. PCR product was cloned using the TOPO TA cloning kit (Clontech), according to the manufacturer's instructions. The PCR product obtained with the universal primer (from the kit) and an MVIG-specific reverse-sense primer 5' TTAGGCTG-GCTAGATCCGGGAAGGAACAG 3' was cloned as described above. Sequences were assembled and aligned using the GCG package.

- **Normalized RT–PCR assay.** An RT–PCR assay was performed as described by Colle et al. (1997). cDNA template and an internal β-actin DNA standard (1 x 10$^4$ copies) were diluted in 10 mM Tris, 50 mM KCl (pH 9 at 25 °C) with 100 µM of each primer, 300 mM of each dNTP and 2.5 U of Taq polymerase (Promega). Amplification required 35 to 40 cycles as follows: 30 s at 94 °C, 30 s at 61 °C and 1 min at 72 °C. Aliquots of each PCR reaction were subjected to digestion with restriction enzymes specific for the wild-type template (BgIII) or the standard template (ClaI) to allow the quantification of β-actin cDNA in the samples. mvig-Specific amplifications were then performed on normalized cDNA samples containing 1 x 10$^4$ β-actin copies. Two pairs of primers specific for mvig were used: MV1forward 5' GCTCGGCTGCTGACCTGTTCC 3' and MV1 reverse 5' AACAAAGCCTGTAGACAGC 3' (amplified product 1157 bp). MV2forward 5' TTAGGTCCGCCCGCCCGCTGCTGCTGCTGACCTGTTCC 3'.
TTGAGGAAG 3’ and MV2reverse 5’ GTCTCTTCCACGTTGAGA-ACG 3’ (amplified product 403 bp). PCR conditions were 94 °C for 5 min, and then 94 °C for 1 min, 56 °C (MV1 primers) or 45 °C (MV2 primers) for 1 min, and 72 °C for 2 min, for 25 to 30 cycles. PCR products were sequenced to verify the specificity of the amplification.

Results

**mvig is induced by VSV and PrV in mouse spleen cells**

We have identified vig1 in trout cells derived from the spleen or the pronephros subjected to incubation with VHSV, a fish rhabdovirus (Boudinot et al., 1999), while Shenk and his group described cig5 in human skin cells subjected to infection with human cytomegalovirus, a herpesvirus (Zhu et al., 1997). We therefore searched for the mouse homologue of vig1 in mouse splenocytes infected with VSV, a rhabdovirus, or PrV, a herpesvirus. Spleen cells were incubated with VSV or PrV, and mvig transcript induction was assessed 5 h later by an RT–PCR assay normalized on the basis of quantitative β-actin expression. Although they were derived from a rat sequence, primer set MV1 amplified a product of the expected size (1157 bp) in samples treated with both viruses and not in the untreated samples (Fig. 1A). A second primer set, MV2, gave a product of the expected size (403 bp) with the same expression pattern (Fig. 1A). In addition we verified that mock-infected spleen cells were also negative (data not shown). These results indicate that the expression of the mouse homologue of vig1 and cig5 (mvig) is also up-regulated by viruses. Both the 1157 bp and 403 bp amplified PCR fragments were cloned and sequenced to assess the specificity of the PCR assay. The derived amino acid sequence was highly similar to vig1/cig5 but lacked the first few amino acids of the protein. The 5’ end of the transcript was amplified by 5’RACE, cloned and sequenced. We thus obtained the complete coding sequence of mvig.

The multiple sequence alignment of mouse, rat, human and trout proteins of the vig1/cig5 family (Fig. 1B) shows that except for the amino-terminal part, these sequences are highly conserved in vertebrates. The carboxy terminus of mvig amino acid sequence (amino acids 69–359) shows 97% identity with best5 (rat) sequence, 92% identity with cig5 (human) and 80% identity with vig1 (trout). The four motifs (I–IV) shared by vig1, best5, cig5 and related bacterial, plant and human sequences (Boudinot et al., 1999) are perfectly conserved in the MVIG protein. Motif I corresponds to the MoaA motif, which is conserved in proteins required for the synthesis of pterin, haem protein. Motif II corresponds to the MoaA motif, which is conserved in proteins required for the synthesis of pterin, haem protein. Motif I corresponds to the MoaA motif, which is conserved in proteins required for the synthesis of pterin, haem protein. Motif II corresponds to the MoaA motif, which is conserved in proteins required for the synthesis of pterin, haem protein.

**mvig is induced in the dendritic cell line D2SC1**

The availability of several established cell lines representative of the different lineages of cells present in the mouse spleen allowed us to investigate the nature of the cells expressing mvig. For this purpose, we performed a series of infection experiments using VSV and PrV and a collection of representative cell lines. The lymphoma EL-4 (Shevach et al., 1972) and the T cell hybridoma B3Z (Shastri & Gonzalez, 1993) represent the T cell lineage. The immature B cell line WEHI-231 (Gutman et al., 1981) and the B lymphoma A20 (Kim et al., 1979) represent the B lineage. A macrophage cell line (P388D1) and dendritic cell line D2SC1 (Paglia et al., 1993; Lutz et al., 1994) were representative of other antigen-presenting cells. The fibroblast line L-929 (ATCC, CCL-1, RF33956) was used as a non-lymphoid/non-myeloid control. The L-929 cell line is known to be permissive to VSV infection.

Fig. 1. The mouse gene homologue of vig1/cig5 is induced by viruses and conserved in vertebrates. (A) RT–PCR assay for mvig expression on cDNA from spleen cells cultured with VSV (lanes 1, 4 and 9), PrV (lanes 2, 5 and 10) or with virus-free medium (lanes 3, 6 and 11). PCR was performed with MV1 primers (lanes 1–3; product size 1157 bp) or MV2 primers (lanes 4–6; product size 403 bp). Samples were normalized on the basis of β-actin expression (lanes 9–11). Size markers were pbr322 digested with Alul (lanes 7 and 12), and phage λ digested with EcoRI and HindIII (lane 8). (B) Multiple alignment of MVIG deduced amino acid sequence (accession no. AF236064) with homologue proteins of rat (BEST5, accession no. Y07704), human (CIG5, accession no. AF026941) and trout (VIG1 accession no. AF076620). Bars indicate the four conserved motifs described in Boudinot et al. (1999).
Each cell line was incubated for 5 h with VSV, PrV or virus-free medium and mvig mRNA accumulation was assessed by an RT–PCR assay normalized on the basis of quantitative β-actin expression. As shown in Fig. 2, mvig induction was observed only in D2SC1. mvig was not induced in mock-infected D2SC1 (data not shown). These results suggest that the induction could be restricted to dendritic cells.

**mvig is selectively expressed in dendritic cells**

To extend the results obtained with the cell lines, we separated fresh mouse spleen cells into dendritic cell-enriched and a dendritic cell-depleted populations using the CD11c magnetic purification protocol from Milteny Biotec. Samples of depleted and enriched cells were subjected to cytofluorometric cell surface analysis to assess the efficiency of the enrichment. The dendritic cell-enriched population was typically > 60% MHC class II++ and > 80% CD11c++, while the dendritic cell-depleted population was less than 4% CD11c++ (Fig. 3A). Dendritic cell-enriched and dendritic cell-depleted populations were then treated for 3 h with PrV, VSV or virus-free culture medium. We observed a high level of mvig expression in the dendritic cell-enriched population, while it was barely detected in the dendritic cell-depleted cells (Fig. 3B). Surprisingly, mvig expression was observed not only in the virus-infected but also in the non-infected dendritic cell-enriched population.

Considering mvig mRNA was not detected in fresh spleen cells (Fig. 1B), this later result probably reflects the activation state of the dendritic cell-enriched preparation. Indeed, the MHC class II++ phenotype of the dendritic cell-enriched population is consistent with an activated state. Moreover, fresh dendritic cells are known to be highly sensitive to stimuli (Gallucci et al., 1999). Even if cells are carefully handled during the staining and separation process, dendritic cells could be set to express activation molecules through cytokines released by activated or damaged neighbouring cells (Gallucci et al., 1999). Regardless, our results finally indicate that mvig induction is restricted to dendritic cells.

**mvig is induced by VSV and PrV in fresh bone marrow-derived dendritic cells**

To further ascertain that mvig is induced by viruses in cells of the dendritic lineage, we used bone marrow-derived dendritic cells (Winzler et al., 1997; Regnault et al., 1999). Briefly, dendritic cells were prepared by culturing bone marrow cells for 10 days in medium supplemented with supernatant of...
**mvig**, a virus-induced gene in dendritic cells

Fig. 4. **mvig** expression in bone marrow-derived dendritic cells. (A) Phenotypic analysis of bone marrow-derived dendritic cells. After 24 h stimulation with LPS (dotted line) or without LPS (solid line), MHC class II, CD40 and B7-2 expression was analysed by FACS analysis with specific and irrelevant antibodies. (B) The expression of **mvig** was determined by RT–PCR assay (with MV2 primers, product size 403 bp) in bone marrow-derived dendritic cells infected with VSV (lane 1) and PrV (lane 2), or with virus-free medium (lane 3). (C) **mvig** expression was also assessed in bone marrow-derived dendritic cells stimulated with LPS for 2 h, 3.5 h and 7.5 h, or before stimulation. All cDNA samples were normalized on the basis of β-actin expression.

GM-CSF-producing fibroblasts. Following this treatment dendritic-specific marker CD11c (N418) was expressed by the bone marrow-derived cells while granulocytic-specific marker GR-1 was not expressed (data not shown), indicating that these cells belong effectively to the dendritic lineage (Fig. 4A). In addition, the majority of cells expressed low levels of MHC class II and B7-2 molecules, and were CD40-negative, indicating an immature state.

To verify that the bone marrow-derived population remained potent, we tested their ability to undergo phenotypic maturation upon activation. They were stimulated for 24 h by LPS and then analysed by flow cytometry for MHC class II, CD40 and B7-2 expression. As shown in Fig. 4 (A, dotted lines), the level of expression of the three markers increased significantly after incubation with LPS. Taken together, these results show that bone marrow-derived cells have the same phenotype as potent, immature dendritic cells residing in non-lymphoid organs.

The bone marrow-derived dendritic cells were subjected to VSV or PrV for 5 h. A strong accumulation of **mvig** transcript was observed in virus-treated cells, while **mvig** transcript was barely detectable in the non-treated cells (Fig. 4B). We also verified that **mvig** is not induced in mock-infected bone marrow-derived dendritic cells (data not shown). These results confirmed that viruses can induce **mvig** in the dendritic lineage.

We have observed **mvig** expression in non-infected CD11c-positive cells obtained by mechanical separation. This result suggested that **mvig** expression may be also linked to a non-specific activation of dendritic cells. To further investigate this point, we searched for **mvig** expression in activated dendritic cells. LPS is a known powerful bacterial activator of dendritic cells. We used LPS to activate bone marrow-derived dendritic cells and then tested for **mvig** expression. Fig. 4 (C) shows that **mvig** was strongly induced in these cells as early as 2 h after activation.

**mvig induction is directly mediated by the VSV particle while the induction by PrV is mediated through the α/β interferon pathway**

We have previously shown that VHSV, a fish rhabdovirus, could induce **vig1** via an interferon-independent pathway, and that a fish interferon-like compound was highly efficient in **vig1** induction (Boudinot *et al.*, 1999). In the murine model, VSV, a mammalian rhabdovirus, seems to share similar properties. Indeed, the up-regulation of **mvig** transcript level in D2SC1 cells was not abolished by addition of CHX, and CHX alone had no effect on the **mvig** mRNA level (Fig. 5A, lanes 2 and 3), indicating that **mvig** induction did not require the de novo synthesis of any protein intermediate. Moreover, both inactivated and live VSV strongly induced **mvig** transcript accumulation in the dendritic cell line D2SC1, showing that this modulation did not require virus replication either (data not shown). To completely exclude the effect of interferon, we infected D2SC1 cells in the presence of anti-mouse α/β interferon antibodies (Fig. 5A, lane 4). While anti-interferon...
antibodies completely abolished \( mvig \) induction by PrV (see below), they had no effect on the \( mvig \) induction obtained in the presence of VSV. We also checked that the anti-interferon antibody used alone cannot induce \( mvig \) (Fig. 5 A, lane 6), and that non-infected cells did not express \( mvig \) (Fig. 5 A, lane 7).

On the contrary, \( mvig \) induction by PrV was completely dependent on the integrity of the interferon pathway. Both CHX and anti-mouse \( \alpha/\beta \) interferon sheep antibodies completely abolished \( mvig \) transcript accumulation induced by PrV in D2SC1 cells (Fig. 5B, lanes 2 and 4, respectively). As a control to this experiment, D2SC1 cells were treated with an irrelevant antibody from sheep, which did not abolish the \( mvig \) induction (Fig. 5B, lane 5). This observation shows that \( mvig \) induction by PrV requires \textit{de novo} protein synthesis and is mediated by the type I interferon pathway.

Taken together, these results clearly indicate that viruses can use either of two distinct pathways to induce \( mvig \). One is independent of the interferon pathway and seems to be mediated directly by the virus particle, the other proceeds through the well-known virus-induced interferon pathway.

**Discussion**

\( vig1 \) and \( cig5 \) are virus-induced genes identified in rainbow trout and humans, respectively (Zhu et al., 1997; Boudinot et al., 1999). We show here that their murine homologue (\( mvig \)) displays a very high sequence similarity with both \( vig1 \) and \( cig5 \) and shares similar characteristics. We also identified mature activated dendritic cells as the main population in which \( mvig \) expression takes place. In these cells, we identified two induction pathways used by viruses, and showed that \( mvig \) expression could also be obtained with LPS, a non-specific activator of bacterial origin.

\( MVG \) protein displays a high sequence similarity with \( VIG1 \) and \( CIG5 \), and shares the conserved motifs identified for \( VIG1 \) and related proteins (Boudinot et al., 1999). The strong conservation of the \( mvig \) family in vertebrates suggests that the function of these genes may be conserved, and therefore should be of importance. This idea is supported by the fact that \( vig1 \) (trout), \( mvig \) (mouse) and \( cig5 \) (human) are induced by viruses.

In the rainbow trout, \( vig1 \) is induced by a fish rhabdovirus, VHSV, either directly or via soluble factors from a conditioned medium containing an interferon-like activity (Boudinot et al., 1999). In humans, \( cig5 \) was described as a cytomegalovirus-induced gene in human primary skin cultures (Zhu et al., 1997). As in the fish, the accumulation of this transcript can be obtained through two pathways, either directly mediated by the virus, or via the type I interferons. We show here that \( mvig \) is also induced through two different pathways. VSV still induces \( mvig \) in the presence of anti-mouse \( \alpha/\beta \) interferon antibodies or CHX, showing that \( mvig \) induction does not require any \textit{de novo} protein synthesis and, specifically, no interferon. Furthermore, mock-infected cells do not express \( mvig \). Although we cannot completely rule out direct \( mvig \) induction through cross-reactive factors released by VSV-producing cells, we consider that direct induction is the most probable hypothesis. Indeed, direct virus induction of the human \( mvig \) homologue was clearly demonstrated (Zhu et al., 1997). While VSV-mediated induction does not require any protein intermediate synthesis, PrV-mediated induction is strictly dependent on interferon. Since anti-mouse \( \alpha/\beta \) antibodies completely abolish the accumulation of \( mvig \) mRNA, it could be inferred there is no alternative to the interferon pathway for PrV. In contrast, Zhu et al. (1997) observed both direct and interferon-mediated induction with another herpesvirus, the cytomegalovirus.

\( mvig \) can be induced in the dendritic cell line D2SC1, in dendritic cells purified from the spleen, and in bone marrow-derived dendritic cells. On the contrary, fibroblast, macrophage or lymphoid cell lines failed to show any accumulation of \( mvig \) transcript. Moreover, \( mvig \) induction was not observed in dendritic cell-depleted spleen cells. These results therefore strongly suggest that \( mvig \) induction by viruses is restricted to cells of the dendritic lineage. Tissue distribution of \( mvig \) homologues is consistent with this hypothesis: \( cig5 \) was induced in human skin primary cultures, which probably contain Langerhans cells (Zhu et al., 1997), and trout \( vig1 \) was expressed in lymphoid organs (Boudinot et al., 1999).

Dendritic cells are professional antigen-presenting cells that are distributed in almost all tissues (Banchereau & Steinman, 1998; Steinman, 1999). They rapidly pervade inflamed tissues, take up antigen, and differentiate upon inflammatory signals to reach a mature activated stage. Mature dendritic cells migrate to lymphatic organs (Sallusto et al., 1998; Sozzani et al., 1998), where they have the unique capacity to prime naive T cells for an antigen-specific immune response (Inaba & Steinman, 1985; Flamand et al., 1994; Porgador & Gilboa, 1995; Zitvogel et al., 1996). Thus, they are likely to play an essential role in the initiation of the specific T helper function and T cytotoxic antiviral responses (Klagge & Schneider-Schaulies, 1999).

Although \( mvig \) function remains enigmatic, its dendritic-targeted induction by VSV and PrV suggests a link with antiviral responses. This idea is reinforced by the conservation of two induction pathways, and by the diversity of the virus families already known to induce the genes of the \( mvig \) family. This redundancy may reflect the general importance of the \( vig1 \) family in the host response to viruses. Interestingly, the direct induction of the \( mvig \) gene family by different viruses (Zhu et al., 1997; Boudinot et al., 1999; this report) is reminiscent of the concept of ‘pattern recognition’ receptors invoked for the initiation of the immune response against evolutionarily distant pathogens (Medzhitov & Janeway, 1997).

In fact, LPS also induces \( mvig \), indicating that the expression of this gene may be a more general feature of pathogen-activated dendritic cells. It is interesting to note that particular interferon-stimulated genes are also involved in the response to bacterial infection. LPS induces a p38-dependent activation
of IRF3, leading to the expression of different interferon-stimulated genes (Navarro & David, 1999). mvig could be considered a member of these genes induced by both interferon and LPS. Finally, the characteristics of the mvig/cig5/vig1 gene family may illustrate the connectivity between several transduction pathways leading to the inflammatory responses.

This work was financially supported by the Institut National de la Recherche Agronomique, France, and by the PRFMMP (Ministère de l’Education Nationale, de la Recherche et de la Technologie, France). S. Salhi received a post-doctoral fellowship from the Institut National de la Recherche Agronomique.

We thank Dr Y. Gaudin for VSV, Dr J.-H. Colle for the internal β-actin DNA standard and Dr P. Ricciardi-Castagnoli for the D2SC1 cell line. We gratefully acknowledge Drs A. Regnault and J. Kanellopoulos for helpful discussions, and Dr S. Matthews for proofreading the manuscript.

P.B., S.R. and S.S. contributed equally to this work.

References


Received 7 June 2000; Accepted 26 July 2000