Virus Infection Switches TLR-3-Positive Human Neurons To Become Strong Producers of Beta Interferon

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To study the capacity of human neurons to mount innate immunity responses to viral infections, we infected cells of a human postmitotic neuron-derivative cell line, NT2-N, with rabies virus (RABV) and herpes simplex type 1 (HSV-1). Changes in neuronal gene expression were analyzed by use of Affymetrix microarrays. Applying a twofold cutoff, RABV increased the transcription of 228 genes, and HSV-1 increased the transcription of 263 genes. The most striking difference between the two infections concerns genes involved in immunity. These genes represent 24% of the RABV-upregulated genes and only 4.9% of the HSV-1-upregulated genes. Following RABV infection, the most upregulated genes belong to the immunity cluster and included almost exclusively genes for beta interferon (*IFN*-β) primary and secondary responses as well as genes for chemokines (*CCL-5*, *CXCL-10*) and inflammatory cytokines (interleukin 6 [IL-6], tumor necrosis factor alpha, interleukin 1 alpha). In contrast, HSV-1 infection did not increase *IFN*-β gene transcripts and triggered the production of only *IL-6* and interferon regulatory factor 1 mRNAs. The microarray results were confirmed by real-time PCR, immunocytochemistry, and enzyme-linked immunosorbent assay. Human neurons were found to express Toll-like receptor 3. They produced *IFN*-β after treatment with poly(I:C) but not with lipopolysaccharide. Thus, human neurons can mount an innate immunity response to double-stranded RNA. These observations firmly establish that human neurons, in absence of glia, have the intrinsic machinery to sense virus infection.

Innate immunity is the first line of defense against invading pathogens. It involves the release of cytokines, including alpha/beta interferons (IFN-α/β) and chemokines; the activation of complement; and the attraction of macrophages, neutrophils, and NK cells into infected tissues. Cells sense viral infection by detecting viral proteins (65) and/or nucleic acids, the double-stranded RNA (dsRNA), a byproduct of the replicative cycles of many viruses, in particular (30). Viral proteins and dsRNA are recognized through receptors such as the evolutionarily conserved Toll-like receptors (TLR) (22, 63). Of the 10 TLRs identified in humans, TLR-3 has been identified to respond to dsRNA (53, 70). The dsRNA can also be sensed by the dsRNA-binding enzyme protein kinase R (PKR) (47) and by the retinoic-induced gene type 1 (RIG-1) (72). Early signaling events initiated by the recognition of virus components include the activation of NF-κB and the phosphorylation of interferon regulatory factor 3 (IRF-3), leading to the production of cytokines (IFN-β, interleukin 6 [IL-6], interleukin 1 alpha [IL-1α], tumor necrosis factor alpha [TNF-α]) and chemokines (CCL-5, CXCL-10, CCL-3, and CCL-4) involved in the initiation or regulation of the inflammatory and antiviral response (1, 58, 60, 72). The interferon response to a viral infection unfolds in two steps (62, 64). The primary response leads to the production of IFN-β. The secondary response results from signaling by the secreted IFN-β through the IFN-α/β receptors (IFN-α/βR) and from the activation of transcription of genes containing IFN-stimulated response elements (ISRE) in their promoters (such as *TLR-3* [48]). Signaling through IFN-α/βR triggers

the activation of Janus kinases and STAT (1 and 2) transcription factors complexed with IRF-9, which stimulate the transcription of *CCL-5* and *CXCL-10* and the expression of genes such as the *IRF-7*, *2′5′OAS*, *MxA*, and *PKR* genes. These last genes all contain binding sites for activated STAT in their promoters. Finally, the upregulation of IRF-7 (and of IRF-1) expression can exert a positive feedback on IFN-β production (62, 64). A scheme of typical primary and secondary IFN-β responses after sensing a viral infection is shown in Fig. 1. IFN-α and -β are important components of immunity, not only because of their antiviral activities but also because they make the link between innate immunity and adaptive immunity (40).

It is still unclear whether the nervous system (NS) can mount an efficient innate immune response during viral attack and which cells of the NS are involved. Neurons can produce chemokines and cytokines: human neurons produce CX3CL-1, IFN-β, IL-6, CCL-5 and CXCL-10 (17, 26, 42) and it was recently found that TLRs are constitutively expressed in the NS (6, 8, 34, 52). Human microglia contains *TLR-2*, *-3*, and *-4* mRNAs (9). One report indicates that human fetal astrocytes express *TLR-3* (9, 21). So far, only mouse cortical neurons have been reported to express TLR (TLR-2) (35), and TLR expression by human neurons has not been observed. Nevertheless, neurons are exclusive targets for infection by some viruses, including rabies virus (RABV) of the genus *Lyssavirus* and herpes simplex virus type 1 (HSV-1) of the *Alphaherpesviridae* subfamily. It would be interesting to know whether neurons have the intrinsic machinery to respond to infection by these viruses by a triggering of an early innate immune response and whether the nature of the innate immune response depends on the type of viral infection.

We analyzed the transcriptome of NT2-N cells, a model of human postmitotic neurons, as triggered by infection with

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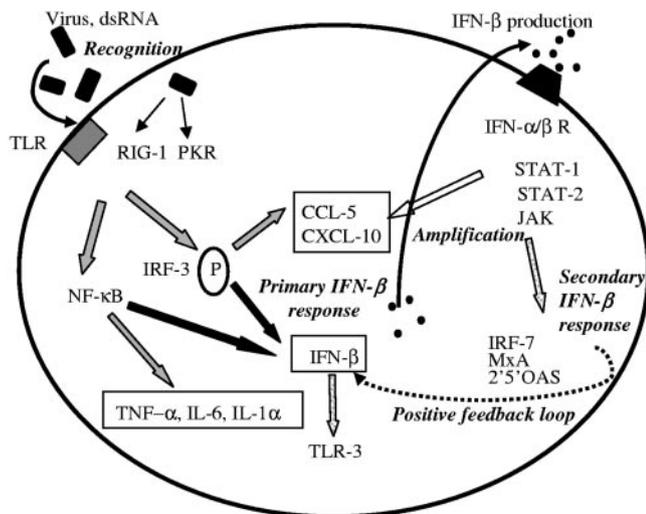† These authors contributed equally to this work.

FIG. 1. Scheme of innate immune responses to virus infection. dsRNA can be sensed (*recognition*) by TLR, dsRNA-binding enzyme PKR, or RIG-1, leading to the activation of NF-κB, the phosphorylation (P) of IRF-3, and the production of inflammatory cytokines (IL-6, IL-1α, TNF-α), chemokines (CCL-5, CXCL-10), and IFN-β. This *primary IFN-β response* is followed by a *secondary response* resulting from the signaling of the secreted IFN-β through IFN-α/βR and from the activation of the transcription of genes containing ISRE in their promoters (including the *TLR-3* gene). Signaling through IFN-α/βR triggers the activation of Janus kinases (JAK) and STAT (-1 and -2) transcription factors, which trigger the expressions of genes such as the *IRF-7*, *2'5'OAS*, *MxA*, and *PKR* genes and the *amplification* of *CCL-5* and *CXCL-10* transcription. Finally, the upregulation of IRF-7 expression can exert *positive feedback* on IFN-β production.

RABV or HSV-1. NT2-N cell cultures are nearly pure populations of terminally differentiated postmitotic cells with biochemical, morphological, and functional similarities to human NS neurons (18, 56, 73). Upon transplantation into the brain, NT2-N cells display characteristics of fully mature human neurons (33, 69). The responses of human neurons to infection by RABV and HSV-1 were analyzed in vitro by using cultures of NT2-N and microarray analysis, coupled to PCR, real-time PCR analysis, immunocytochemistry, and enzyme-linked immunosorbent assay (ELISA).

We report that human neurons express TLR-3 and can mount differing innate immune responses after RABV and HSV-1 infections. This work provides new insights into the role of neurons in the defense of the NS against infection.

MATERIALS AND METHODS

Human neurons and virus. Human NT2-N cells (56) were obtained from Ntera-2clD/1 cells (ATCC CRL, 1973) and differentiated according to a modification of the original procedure subsequently modified as described previously (15, 54). The laboratory RABV strain CVS (ATCC vr959), a highly pathogenic strain of the virus causing fatal encephalomyelitis in mouse after intramuscular injection (10), was propagated as previously described (66). HSV-1 strain KOS (61) was propagated in U373MG cells.

Analysis of gene expression after RABV or HSV-1 infection. NT2-N cells were mock infected or infected with either RABV or HSV-1. RNAs were isolated 24 h (RABV) or 18 h (HSV-1) later from both infected and mock-infected cultures by use of an RNeasy kit. Duplicate samples obtained 2 weeks apart were used to minimize experimental variations. Control quality was monitored on Agilent RNA Nano LabChips. Gene expression profiles were analyzed using Affymetrix microarrays for the human genome (U133A and -B) containing probe sets representing 38,903 transcripts. Experiments were done at the Génopole Stras-

bourg-Alsace-Lorraine (http://www-genopole.u-strasbg.fr/) by Affymetrix standard protocols. Detected probe sets were selected according to the "presence calls," and the changes (*n*-fold) were established by Affymetrix software (Microarray Suite v5.0 and Data Mining Tool v2.0). Only significantly changed probe sets ($P < 0.05$) were considered. Upregulated genes were distributed into clusters of cell functions using NetAffyx Gene Ontology Mining Tool software.

Standard and real-time RT-PCR analysis and hybridization. RNA was extracted 1, 6, and 24 h after RABV infection and 24 h after treatment with IFN-β, poly(I:C), or lipopolysaccharide (LPS) with RNeasy kits. RNA quality was monitored on Agilent RNA Nano LabChips. cDNA synthesis was performed with 1 μg RNA by using oligo(dT) primers (100 ng) (Table 1). Superscript II reverse transcriptase (RT) and *Taq* DNA polymerase were used for RT-PCR in a Px2 thermal cycler. There were 30 cycles of amplification, performed as follows: 4 min at 94°C, 1 min at 60°C, 1 min at 72°C, 10 min at 72°C, and, finally, cooling. 18S RNA was used as a reference (housekeeping gene). Real-time RT-PCR analysis was performed with an ABI Prism 77700 sequence detection system. Methods and relative quantifications of gene expressions were performed using the comparative method according to the manufacturer's instructions.

Immunocytochemistry. Infected and noninfected NT2-N cells were washed once with phosphate-buffered saline (PBS) containing $Ca^{2+}Mg^{2+}$, fixed with 4% paraformaldehyde for 30 min at room temperature (Rt), washed again, and treated with gelatin (1% in water) for 5 min at 4°C. The samples were then incubated for 30 min at Rt in 0.3% Triton X-100-PBS, and the surface immunoglobulin (Ig) receptors were blocked with a saturating medium (2% bovine serum albumin and 5% fetal calf serum in PBS) for 30 min at Rt and for 10 min with Fc-block (1 mg/10⁶ cells). Viral antigens were detected by incubation with HSV-1 (early antigens) or RABV (nucleocapsid antigens) fluorescein isothiocyanate (FITC)-conjugated rabbit antibody (Ab) for 30 min at Rt. Rabbit Ab directed against NeuF-H, mouse Ab specific for human CXCL-10, for Tau, or for synaptophysin, and rabbit Ab directed against human TLR-3 were incubated with the samples for 1 h at Rt, and then biotinylated anti-rabbit Ab, FITC-conjugated anti-mouse Fab'2, biotinylated anti-mouse IgG, or Cy5-conjugated anti-rabbit Ab was added for 30 min at Rt. Slides were also incubated for 30 min at Rt with peroxidase-conjugated streptavidin for Tau and synaptophysin detection, a procedure followed by 6 min of incubation with diaminobenzidine or with Cy3-conjugated streptavidin for the detection of NeuF-H. Nuclei were stained with Hoechst 33342 or hematoxylin. Ab and reagents were diluted in saturating medium. Slides were washed with PBS $Ca^{2+}Mg^{2+}$, except for the last wash, which was done with water. Coverslips were mounted in Fluoromount-G, and the samples were observed under a Leica DM 5000B UV microscope equipped with a DC 300FX camera. Images were processed with the Leica FW 4000 software.

IFN-β ELISA. Human IFN-β was detected in NT2-N culture supernatants by enzyme immunoassay following the manufacturer's instructions.

Reagents and antibodies. FITC-conjugated rabbit anti-HSV-1 protein Ab was from Dako. FITC-conjugated rabbit anti-RABV nucleocapsid was from Bio-Rad. Anti-human CXCL-10 monoclonal Ab 6D4 was from Abcam. The anti-human TLR-3 Ab, H125 (rabbit polyclonal Ab), was from Santa Cruz Biotechnology. Anti-bovine Tau mouse IgG (which also recognizes human Tau) was from Boehringer. The human IFN-β ELISA kit used was from PBL Biomedical Laboratories. Rabbit anti-neurofilament 200, NeuF-H, anti-rat synaptophysin mouse IgG (which reacts with human synaptophysin), poly(I:C), LPS, and the 3,3'-diaminobenzidine tablets, Sigma Fast-DAB, were from Sigma Chemical Corp. Biotinylated anti-rabbit Ab was obtained from Vector Laboratories. Biotinylated anti-mouse IgG and peroxidase-conjugated streptavidin were from Amersham Life Sciences. Cy3-conjugated streptavidin and FITC-conjugated Fab'₂ anti-mouse Ab were from Jackson Immunoresearch Laboratories. Recombinant human IFN-β (Betaferon) was from Schering. Fluoromount-G was from Southern Biotechnology Associates. The RNeasy Protect kit used was from QIAGEN. Molecular weight markers, *Taq* polymerase, and phiX 174 DNA/HaeIII were from Promega. Superscript II RT was from Invitrogen. oligo(dT) primers were from Eurogentec. Agilent RNA Nano LabChips were from Agilent Technologies. Human genome U133A and -B arrays were from Affymetrix. Human brain total RNA was from BD Biosciences.

RESULTS

Human NT2-N cultures are pure neuron cultures susceptible to HSV-1 and RABV infection. NT2-N cells progressively develop an extensive network of neurites (Fig. 2). More than 95% of the cells expressed the high-molecular-weight neurofilament protein NeuF-H, as assayed by immunocytochemistry

TABLE 1. List of primers

Gene product or gene	Full name and/or description	Primer sequence (5′→3′)[a]
IFITI	Interferon-induced protein 1	F: GCCACAAAAAATCACAAGCCA
		R: TCCATTGTCTGGATTTAAGCGG
CCL-5	Chemokine ligand 5 (RANTES)	F: CGG CAC GCC TCG CTG TCA TC
		R: GCA AGC AGA AAC AGG CAA AT
IFN-β	Beta interferon	F: GCC GCA TTG ACC ATC TAT GAG A
		R: GAG ATC TTC AGT TTC GGA GGT AAC
CCL-3	Chemokine ligand 3 MIP-1 alpha	F: TGG CTC TCT GCA ACC AGT TCT
		R: GTA GCT GAA GCA GCA GGC G
CCL-4	Chemokine ligand 4 MIP-1 beta	F: TGT CCT GTC TCT CCT CA
		R: CAT TGG TGC TGA GAG CG
CXCL-11	Chemokine ligand 11 (I-TAC)	F: GCA GTG AAA GTG GCA GA
		R: GAT TTA GGC ATC GTT GTC C
CXCL-10	Chemokine ligand 10 (1P-10)	F: TGA GCC TAC AGC AGA GGA A
		R: TAC TCC TTG AAT GCC ACT TAG A
OAS-1	2′,5′-oligoadenylate synthetase 1	F: TCA GAA GAG AAG CCA ACG TGA
		R: CGG AGA CAG CGA GGG TAA AT
CCL-20	Chemokine ligand 20 (LARC)	F: GATGTCAGTGCTGCTACTCCACC
		R: TGTGTATCCAAGACAGCAG
CiG-5	Viperin	F: GGGCAAGTTGGTGAGGTTCTG
		R: CCGGATCAGGCTTCCATTG
ISG20	Interferon-stimulated gene (20 kDa)	F: AGAGTGGCCTGGCTCGTTG
		R: GGCCGGATGAACTTGTCGT
PTGS2	Cox2	F: CCCCAGGGCTCAAACATGAT
		R: AGCTGGCCCTCGCTTATGAT
NeuF-H	Neurofilament H protein	F: CCCCAGGCGATGGACAATTATGAT
		R: CACTTGGTTTTATTGCACAGAAGC
STAT-1	Signal transducer and activator of transcription 1	F: TTCTGTGTCTGAAGTGTAAGTGAA
		R: TAACACGGGGATCTCAACAAGTTC
IRF-7	Interferon regulatory factor 7	F: CAGCTGCGCTACACGGAGGAACTG
		R: CTCCAGCTCCATAAGGAAGCACTC
NF-κB	Nuclear factor of kappa light polypeptide gene enhancer	F: GATGAAGATTGAGCGGCCTGTAAC
		R: TCCTCCGCTTCCGCTGCACCTCTT
TLR-3	Toll-like receptor 3	F: AAC GAC TGA TGC TCC G
	TaqMan	R: CCA GAG CCG TGC TAA G
	PCR	F: GAGGCGGGTGTTTTTGAACTAGAA
		R: AAGTCAATTGTCAAAAATAGGCCT
MOG	Oligodendrocyte-myelin glycoprotein	F: GAAAGCTGGGCAACATGCCTGCTT
		R: TGTCACTCCGGCTAGAGTGCAGTG
CD200R	CD200 receptor	F: TTAACACTTCATGGCCTGTAAAGA
		R: TGTGCCATTGCTCCAGTATTCTTG
TNF-α	Tumor necrosis factor alpha	F: GGCATCCTGAAGTATGTCACATAG
		R: CATTAGCAGCAACACCAGAAAAAT
IL-6	Interleukin 6	F: ATGCCAGCCTGCTGACGAAGCTGC
		R: TATTTGAGGTAAGCCTACACTTTC
RIG-1	Retinoic-induced gene-1	F: GAGTGTCTTTTCTTATGTGATTTT
		R: GCAGGCAAGTCTTACATGGCAGCA
18S	Ribosomal 18S	F: CTT AGA GGG ACA AGT GGC G
		R: ACG CTG AGC CAG TCA GTG TA
N	Rabies N protein	F: GGA ATT CTC CGG AAG ACT GCA CCA GCT ATG G
		R: AGA ATT CCC ACT CAA GCC TAG TGA ACG G
UL54	HSV-1 protein	F: CGC CAA GAA AAT TTC ATC GAG
		R: ACA TCT TGC ACC ACG CCAG

[a] F, forward; R, reverse.

and RT-PCR (Fig. 2A). NT2-N cells also expressed the microtubule-associated Tau protein, a marker of the late stage of neuronal differentiation essential for maintaining the cytoskeleton and axonal transport (Fig. 2A). They also expressed synaptophysin, a synaptic vesicle protein involved in neuronal transmission (Fig. 2A). Differentiation of Ntera-2clD/1 did not give rise to any glial cells, as tested by RT-PCR, and mRNAs that are markers of astrocytes (glial fibrillary acidic protein [GFAP]), oligodendrocytes (oligodendrocyte-myelin glycopro-

tein [MOG]), and microglia (the CD200 receptor [CD200R]) (2, 59, 71) were all absent (Fig. 2A; PCR).

Following infection, HSV-1 and RABV proteins were clearly detected by immunocytochemistry (Fig. 2B), and the HSV-1 *UL54* gene and RABV *N* gene amplicons were detected by RT-PCR (PCR results shown in Fig. 2B). Thus, NT2-N cells were susceptible to infections by HSV-1 and RABV.

Transcriptional programs of human neurons activated during RABV and HSV-1 infection. We searched for neuronal
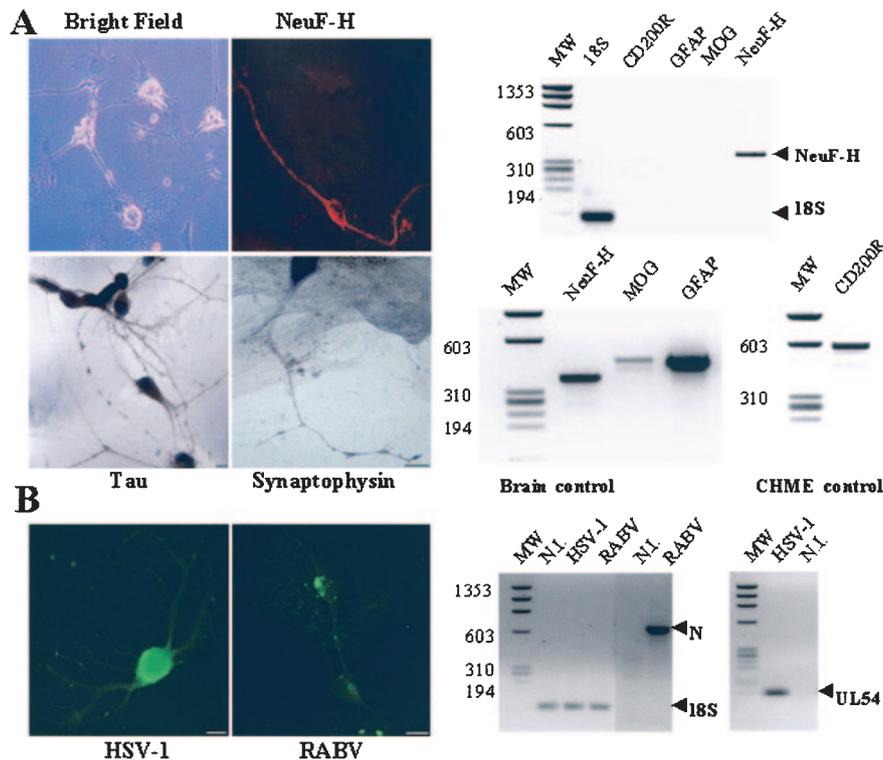
FIG. 2. NT2-N cultures are pure human postmitotic neuron preparations susceptible to RABV and HSV-1 infection. (A) NT2-N cultures are pure cultures of neurons. Shown is a bright-field, phase-contrast photomicrograph of NT2-N culture (magnification, ×20). NT2-N cells express the high-molecular-weight neurofilament (NeuF-H); Tau, a marker of highly differentiated neurons (immunocytochemistry and/or RT-PCR); and synaptophysin, a marker of synaptic vesicles (immunocytochemistry). In contrast to human brain cells, they do not express *MOG* or *GFAP* markers of oligodendrocytes or astrocytes, respectively, nor *CD200R*, a marker of microglia, expressed here by the human microglial cell line CHME (31). (B) After a 24-h infection, NT2-N cells express HSV-1 and RABV antigens in cell bodies and neurites. Transcripts of HSV-1 (*UL54* gene) and RABV (*N* gene) can be detected in NT2-N cultures infected for 24 h (PCR). MW, molecular weights. Bars in the photographs represent 10 μm.

genes for which transcription is upregulated during RABV and HSV-1 infection by transcriptome analysis using Affymetrix microarrays U133A and U133B. RNAs were prepared from two batches of cultures of NT2-N cells that were either not infected (24 h and 18 h) or were infected with RABV (24 h) or with HSV-1 (18 h). The percentages of NT2-N cells infected were the same in the two infections (80% of each culture was infected, as detected by immunochemistry). A cutoff of a two-fold increase was used to identify upregulated genes by microarray analysis: RABV infection upregulated the expression of 228 genes, and HSV-1 upregulated the expression of 263 genes. The genes were distributed into 10 clusters defined by the NetAffx gene ontology mining tool software, and this suggested that RABV-1 and HSV-1 affected cell physiology in very different ways (Fig. 3A). RABV upregulated 56 genes of the immunity cluster of genes (24% of all upregulated genes), whereas HSV-1 upregulated only 13 genes in this cluster (4.9%) (Fig. 3B). RABV upregulated only 5 genes involved in neurogenesis/neurotransmission (2.0% of all genes upregulated by RABV infection), whereas HSV-1 upregulated 37 of the genes of this cluster (14%). A list of the 56 immunity genes upregulated by a 24-h RABV infection is shown in Table 2 in order of overexpression magnitude (the greatest increase was 328.5). These genes include the *IFN-β* (×150) gene; the genes coding for chemokines *CCL-5* (×232), *CCL-3* (×105), *CCL-4* (×97), *CCL-20* (×42), *CXCL-9* (×12), *CXCL-10* (×61),

CXCL-11 (×86), and *CXCL-3* (×3.2); several genes whose expressions are under the control of IFN-β, notably, those coding for *IFIT-1* (×328.5), *IFIT-2* (×39), *IFIT-4* (×72) *ISG-20* (×37), *GBP-5* (×55), *GBP-1* (×11), *2′5′OAS-1* (×43.0), *2′5′OAS-3* (×6.1), and *MxA* (×15.0); genes coding for the interferon regulatory factors IRF-7 (×7.3) and *IRF-1* (×3.8); genes coding for the activators of transcription *STAT-1* (×8.2), *STAT-2* (×4.0), and *NF-κB* (×2.1); and genes which can sense dsRNA, in particular *PKR* (×2.6), *RIG-1* (×3.6), and *TLR-3* (×2.0). RABV upregulated a factor of the alternate pathway of complement, *factor B* (×10.0). It also increased the expressions of genes for inflammatory cytokines *IL-6* (×8.5), *IL-1α* (×4.3), *TNF-α* (×2.4), and *IL-15* (×2.0); of the *HLA-E* (×2.1) gene coding for the molecule of the nonclassical histocompatibility gene of class I that binds leader peptides from other class I molecules and CD94/NKG2, a receptor expressed by natural killer cells (12). RABV also stimulated the expression of *Ly-6E*, also called *CD107* (×2.2), a marker involved in T-cell adhesion and activation, the expression of which is induced by IFN in neuronal cells (19).

The 25 genes for which expression was most strongly stimulated were all genes involved in the innate immunity response.

HSV-1 upregulated only 2 of the 56 immunity genes upregulated by RABV: those coding for IL-6 and IRF-1. The increases were only moderate (×3.2 and ×2.05, respectively)
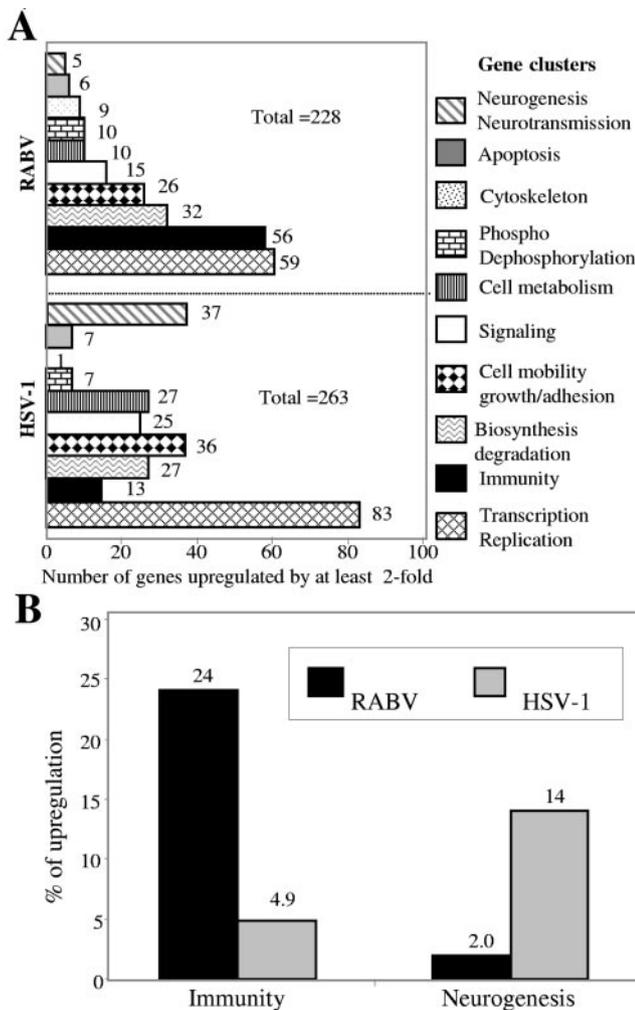
FIG. 3. Patterns of genes upregulated by RABV and HSV-1. (A) Numbers of genes upregulated by at least twofold after RABV and HSV-1 infection in each of the 10 gene clusters defined by the Affymetrix annotation program. HSV-1- and RABV-infected transcriptomes differ in terms of the clusters of genes involved with the cytoskeleton and in neurogenesis/neurotransmission, cell metabolism, and immunity. (B) Distribution (percentages) of upregulated genes into immunity and neurogenesis/neurotransmission clusters after RABV or HSV-1 infection. RABV upregulates mainly genes of immunity, whereas HSV-1 mainly upregulates genes involved in neurogenesis.

(Table 2). Thus, in striking contrast to RABV infection, HSV-1 infection did not increase the abundance of *IFN*-β transcripts or of any of the transcripts of the IFN response genes. HSV-1 specifically stimulated the transcription of genes belonging to the immunity cluster which were not increased by RABV, for example, those coding for *CXCR-4* (×7.9), *CXCL-12* (×4.05), and *CD80*, a costimulatory adhesion molecule (×3.05).

Of the 10 TLR present on an Affymetrix U133 array, only *TLR-1*, *TLR-2*, *TLR-3*, and *TLR-4* could be detected in NT2-N (Table 3). RABV infection, but not HSV-1 infection, upregulated the expression of *TLR-3* only (×2.0). This observation suggests that, among the TLRs, NT2-N cells have the capacity to express those which react with dsRNA (*TLR-3*) and LPS (*TLR-4*).

We used PCR to validate the expression patterns detected by using microarrays in RABV-infected NT2-N cells. Cultures of NT2-N cells were infected for 24 h with RABV, RNA was extracted, and mRNAs of particular genes were studied by RT-PCR and quantified by real-time RT-PCR using 18S mRNA as a control (Fig. 4A). Fourteen genes were chosen as representative either of the early innate immunity response (the genes for *IFN*-β and the chemokines *CCL-5* and *CXCL-10*) or of inflammatory cytokines (*TNF*-α) and interferon-regulated genes (*PTSG*, *ISG-20*, *CiG-5*, *2'5'OAS*, *TLR-3*). PCR and real-time PCR analysis confirmed the increases of the expressions of these genes (Fig. 4A for PCR and Fig. 4B for real-time PCR with representative genes, such as those coding for *2'5'OAS-1*, *PTSG-20*, *IFN*-β, *IFIT-1*, and *CCL-5*). Uninfected neurons expressed *IFIT-1*, *CCL-4*, *CXCL-10*, *2'5'OAS-1*, *CCL-20*, *ISG-20*, *TLR-3*, and *TNF*-α but not *CCL-5*, *IFN*-β, *PTGS-2*, or *CiG-5*. The absence of transcription in control cells may account for the strong relative amplification of the *IFN*-β or *CCL-5* genes after RABV infection, as observed both by microarray analysis (×150 and ×232, respectively; Table 2) and by real-time PCR analysis (×700 and ×52,000, respectively; Fig. 4B). We specifically confirmed the absence of *IFN*-β transcripts after 6 and 24 h in HSV-1-infected cultures (data not shown).

Thus, the expression of *IFN*-β, *inflammatory cytokines/chemokines*, and *IFN*-β-regulated genes is activated in NT2-N cells following RABV infection. This reaction is specific to RABV and is not observed after HSV-1 infection. NT2-N cells react to HSV-1 infection by a more limited innate immune response involving the stimulation of only a few innate immunity genes, including the *IL-6*, *IRF-1*, and *CXCL-12* genes but excluding *IFN*-β and *IFN*-β-regulated genes.

RABV-infected NT2-N cells produce CXCL-10 and TLR-3 proteins and secrete IFN-β. We used immunocytochemistry (CXCL-10 and TLR-3) and immunoassay (IFN-β) to demonstrate that the activation of gene transcription led to the production of IFN-β, CXCL-10, and TLR-3 proteins.

The accumulation of CXCL-10 was clearly demonstrated in the cytoplasms of NT2-N cells infected with RABV for 24 h, whereas only traces of the protein were detected in uninfected cells (Fig. 5A), consistent with the transcription level (Fig. 4).

TLR-3 accumulated in NT2-N cells as cytoplasmic and perinuclear inclusions and at the membrane peripheries (arrows in Fig. 5B) in both uninfected NT2-N cells (panel a in Fig. 5B) and in NT2-N cells infected with RABV for 24 h (panel b in Fig. 5B). The intensity of TLR-3 staining in intracytoplasmic vesicles was however stronger in RABV-infected cells (compare panels a and b in Fig. 5B). This is consistent with the transcription results: *TLR-3* mRNAs were detected by RT-PCR in uninfected neurons but were more abundant following RABV infection (Fig. 4A).

Supernatants of mock-infected HSV-1 and RABV-infected NT2-N cells were harvested 6, 24, and 48 h after RABV infection and 18 h after HSV-1 infection. They were assayed for the presence of IFN-β by immunoassay (Fig. 5C). IFN-β was detected in the supernatant of NT2-N cells 6 h, 24 h, and 48 h following RABV infection. IFN-β production peaked at 24 h and declined thereafter, suggesting that IFN-β is secreted transiently. In contrast, IFN-β was not detected in the supernatants of mock-infected NT2-N cells or in the 18-h supernatant of

TABLE 2. Immunity genes upregulated by RABV

Gene product or gene[a]	Full name and/or description	Accession no. (Affymetrix)	Fold RABV (24 h)[b]	Fold HSV-1 (18h)**
IFIT-1	Interferon-induced protein with tetratricopeptide repeats 1	203153	328.5	0
CCL-5	Chemokine (C-C motif) ligand 5	1405	232.0	0
IFN-β	Beta interferon	208173	150.0	0
CCL-3	Chemokine (C-C motif) ligand 3	205114	105.0	0
CCL-4	Chemokine (C-C motif) ligand 4	204103	98.0	0
CXCL-11	Chemokine (C-X-C motif) ligand 11	211122	86.0	0
IFIT-4	Interferon-induced protein with tetratricopeptide repeats 4	229450	72.0	0
CXCL-10	Chemokine (C-X-C motif) ligand 10	204533	61.0	0
GBP-5	Guanylate binding protein 5	238581	55.0	0
PTGS-2	Prostaglandin-endoperoxide synthase 2 (COX-2)	204748	50.0	0
OAS-1	2′,5′-oligoadenylate synthetase 1	205552	43.0	0
CCL-20	Chemokine (C-C motif) ligand 20	205476	42.0	0
IFIT-2	Interferon-induced protein with tetratricopeptide repeats 2	226757	39.0	0
CIG-5	Viperin	213797	38.0	0
ISG-20	Interferon-stimulated gene, 20 kDa	204698	37.0	0
GIP-2	Interferon, alpha-inducible protein (clone IFI-15K)	205483	28.0	0
Mx-A	Myxovirus (influenza virus) resistance protein type A	202086	15.0	0
IFI-27	Interferon, alpha-inducible protein 27	202411	15.0	0
IFITM-1	Interferon-induced transmembrane protein 1 (9–27)	214022	13.5	0
CXCL-9	Chemokine (C-X-C motif) ligand 9	203915	12.0	0
IFI644	Interferon-induced protein 44	214453	11.0	0
BF	Factor B alternative complement pathway	202357	10.0	0
GIP-3	Interferon, alpha-inducible protein (clone IFI-6-16)	204415	10.0	0
GBP-1	Guanylate binding protein 1, interferon inducible	202269	10.5	0
IL-6	Interleukin 6 (beta interferon 2)	205207	8.5	2.05
STAT-1	Signal transducer and activator of transcription 1	209969	8.2	0
IRF-7	Interferon regulatory factor 7	208436	7.3	0
DnaJB5	DnaJ (Hsp40) homolog, subfamily B, member 5	207453	7.0	0
SAMHD-1	SAM domain and HD domain 1	204502	6.5	0
OAS-3	2′,5′-oligoadenylate synthetase 3	218400	6.1	0
IFIT-5	Interferon-induced protein with tetratricopeptide repeats 5	203596	6.1	0
OAS-2	2′,5′-oligoadenylate synthetase 3, 100 kDa	204972	5.2	0
INDO	Indoleamine-pyrrole 2,3 dioxygenase	210029	4.4	0
IL1-α	Interleukin 1 alpha	210118	4.3	0
STAT-2	Signal transducer and activator of transcription 2, 113 kDa	225636	4.0	0
IRF-1	Interferon regulatory factor 1	202531	3.8	3.2
ISGF-3G	Interferon-stimulated gene factor 3 gamma, 48 kDa	203882	3.8	0
IF-35	Interferon-induced protein 35	209417	3.6	0
RIG-1	Retinoic acid-inducible gene 1 (DEAD/H box polypeptide)	242961	3.6	0
TAP-1	Transporter 1, ATP binding cassette, subfamily B (MDR/TAP)	202307	3.4	0
CIS	Complement component 1, S subcomponent	208747	3.3	0
CXCL-3	Chemokine (C-X-C motif) ligand 3	207850	3.2	0
TAP-2	Transporter2, ATP-binding cassette, subfamily B (MDR/TAP)	204770	3.1	0
PTX-3	Pentaxin-related gene, rapidly induced by IL-1 beta	206157	2.9	0
IF-16	Interferon, gamma-inducible protein 16	208965	2.8	0
NCF-2	Neutrophil cytosolic factor 2	209949	2.6	0
PKR	Interferon-inducible double-stranded RNA-dependent protein kinase	204211	2.6	0
NOD27	Nucleotide-binding oligomerization domain 27	226474	2.5	0
GBP-3	Guanylate binding protein 3	223434	2.5	0
TNF-α	Tumor necrosis factor alpha	223501	2.4	0
PTGER-4	Prostaglandin E receptor 4 (subtype EP4)	204897	2.4	0
Ly-6E	Lymphocyte antigen 6 complex locus E (CD107)	202145	2.2	0
NF-κB2	Nuclear factor kappa-B2	207535	2.1	0
HLA-E	Major histocompatibility complex, class 1, E	200904	2.1	0
TLR-3	Toll-like receptor 3	206271	2.0	0
IL-15	Interleukin 15	205992	2.0	0

[a] Underlined genes and gene products are those for which transcriptions were verified by PCR (see Fig. 4).
[b] Transcripts that were more than twofold more abundant in NT2-N cultures infected by RABV for 24 h than in mock-infected NT2-N cultures.
[c] Increases (*n*-fold) of the same transcripts in NT2-N cultures infected by HSV-1 for 18 h. In addition to those for IL-6 and IRF-1, which were activated both by RABV and HSV-1, HSV-1 specifically amplified the expressions of 11 other immunity genes/gene products: *EDA*, Affymetrix identification number (AIN) 211128 (×10.5); *CXCR-4*, AIN 217028 (×7.9); *CXCL-12*, AIN 203666 (×4.5); *CD80*, AIN 207176 (×3.05); *CEBPE*, AIN 214523 (×3.65); *PGLYRP4*, AIN 220944 (×2.8); *SCUBE-1*, AIN 233988 (×4.4); *LILRB-4*, AIN 210152 (×3.4); *CEACAM-1*, AIN 211883 (×3.4); *C4A/B*, AIN 214428 (×3.9); and *ULBP-1*, AIN 221323 (×2.5).

NT2-N cells infected with HSV-1, consistent with the results of the transcription analyses.

These findings establish that NT2-N cells produce TLR-3 protein and that RABV infection increases the amount pro-

duced. Immunocytochemistry analysis suggested that TLR-3 trafficking may be modified following infection. RABV-infected NT2-N cells secrete IFN-β and produce CXCL-10 protein. Thus, at least for these three genes, transcriptional in-

TABLE 3. NT2-expressed *TLR-1*, *-2*, *-3*, and *-4*

Gene	ProbeSet no.	Presence[a]	P value
TLR-1	210176	+	0.030273
TLR-2	204924	+	0.037598
TLR-3	206271	+	0.000732
TLR-4	221060	+	0.023926
TLR-5	210166	−	0.219482
TLR-6	207446	−	0.533936
TLR-7	220146	−	0.633789
TLR-8	220832	−	0.725830
TLR-9	223903	−	0.466064
TLR-10	223750	−	0.432373

[a] Presence (+) of *TLR* is indicated when the *P* value is lower than 0.02; the presence is uncertain (−) when the *P* value is higher than 0.02.

creases observed by microarrays and PCR analysis are associated with increased protein production.

Kinetics of innate immune response in RABV-infected NT2-N cells. We used real-time RT-PCR to document the time course of the IFN-β response in RABV-infected NT2-N cells and also to identify the cascades of genes involved in the innate immune response. IFN-β gene transcripts and the transcripts of six other genes were analyzed by real-time RT-PCR at 1, 6, and 24 h postinfection. The other six genes were the *CCL-5* gene, the transcription of which is modulated by the phosphorylation of IRF-3; the *2'5'OAS-1* and *IRF-7* gene, the expressions of which are controlled by STAT-1 and -2; the *TLR-3* gene, which can sense RNA viral infection; and the *NF-κB* and *STAT-1* genes, coding for transcription factors. Transcripts of the *TNF-α* and *IL-6* genes, which are upregulated following NF-κB activation or phosphorylation of IRF-3, were also analyzed by RT-PCR. The *CCL-5* and *IFN-β* genes were the first genes to be upregulated: their transcripts were more than 10-fold more abundant in infected neurons than in uninfected neurons 1 h after RABV infection (Fig. 6A). The transcription of the *IFN-β* gene reached a maximum 6 h postinfection and then decreased. The early increases in the transcriptions of the *IFN-β* and *CCL-5* genes was followed by increased transcriptions of the *2'5'OAS-1*, *IRF-7*, and *STAT-1* genes, which reached 10 times the control values 6 h after infection. *TNF-α* and *IL-6* gene transcripts were found 6 h postinfection, and that of the *IL-6* gene was found even 1 h after infection (Fig.
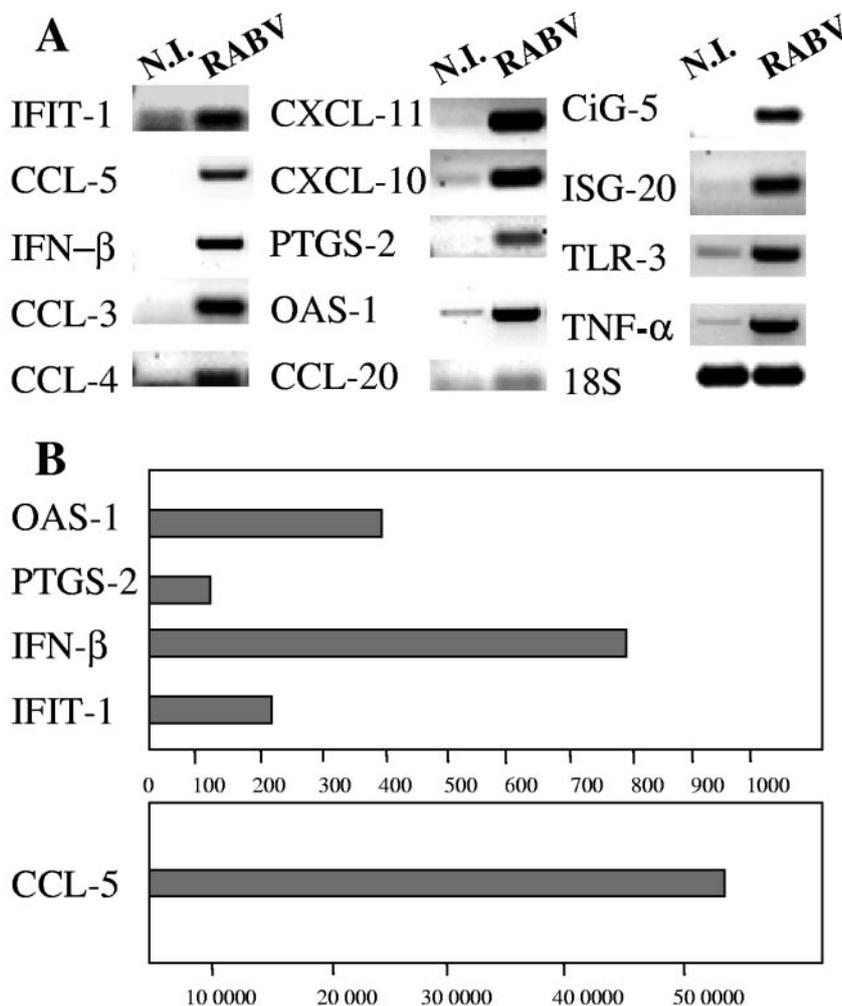

FIG. 4. PCR confirmation of the differential gene expression detected by microarray analysis. (A) PCR in 24-h noninfected (N.I.) and RABV-infected NT2-N cells. 18S was used as a reference housekeeping gene. (B) Real-time PCR. Results are expressed as multiples of the noninfected NT2-N value (defined as 1). Error for the measure was less than 10%.
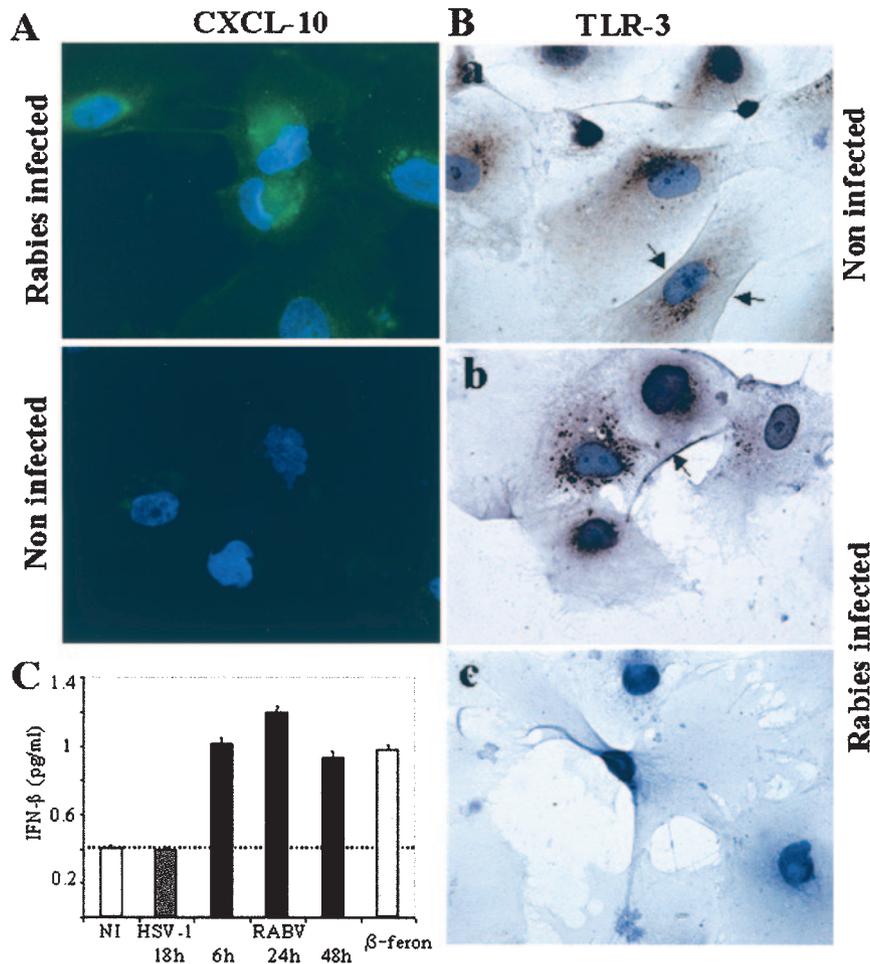
FIG. 5. RABV-infected NT2-N cells express CXCL10 and TLR-3 and secrete IFN-β. (A) CXCL-10 was detected by immunofluorescence in 24-h RABV-infected NT2-N cultures, but only trace or no CXCL-10 was present in noninfected NT2-N cultures. (B) With a polyclonal rabbit Ab directed against human TLR-3, TLR-3 protein was specifically detected by immunocytochemistry in noninfected (a) and RABV-infected (b) cultures. (c) The TLR-3 Ab was omitted. TLR-3 accumulates in intracytoplasmic vesicles (brown coloration) and at the membrane (arrows). Nuclei (blue) are stained with hematoxylin. (C) Detection of human IFN-β by ELISA in the supernatants of noninfected and 6-, 12-, 24-, and 48-h RABV- or 18-h HSV-1-infected NT2-N cultures. Betaferon was used as a positive control.

6A). The last genes to have transcription upregulated (24 h or later) were the *RIG-1*, *TLR-3*, and *NF-κB* genes (Fig. 6A).

Thus, during the 24 h following RABV infection, (i) *CCL-5* and *IFN-β* genes are transcribed 1 h after infection; (ii) this transcription is followed, after 6 h, by the upregulation of *IL-6*, *TNF-α*, *2′-5′OAS-1*, *IRF-7*, and *STAT-1* gene transcription; and (iii) the transcriptions of the *RIG-1*, *NF-κB* and *TLR-3* genes are upregulated later (24 h). The increased transcription of the *IFN-β* gene is transient. These results suggest that the transcriptome analysis performed 24 h after RABV infection reflects both the triggering of *IFN-β* and *CCL-5* in the first hour of infection and a subsequent cascade of events triggered by the early expression of these two mediators.

Treatment of NT2-N cells with human recombinant IFN-β increased the responses of genes which regulate the secondary IFN-β response genes but did not amplify IFN-β gene transcription. IFN-β is present in the supernatant of RABV-infected NT2-N cultures, and we therefore tested whether the addition of IFN-β to NT2-N cultures triggers the cascade of

events upregulated by IFN-β (Fig. 1). Uninfected NT2-N cells were treated (or mock treated) with 1,000 IU of recombinant IFN-β (rIFN-β) for 24 h, and the expressions of the *CCL-5*, *CXCL-10*, *TLR-3*, and *IRF-7* genes were compared by real-time RT-PCR (Fig. 6B). The transcriptions of the *CXCL-10*, *CCL-5*, *IRF-7*, and *TLR-3* genes were increased by this treatment (increases of 1,965-, 71-, 42-, and 26.5-fold, respectively), indicating that exogenous IFN-β triggered in NT2-N cells a cascade of events compatible with a secondary IFN-β response. In particular, these events include the upregulation of *TLR-3*, the promoter region of which contains an ISRE susceptible to IFN-α/β. Thus, *TLR-3* upregulation in human neurons may be controlled by IFN. The effect of exogenous IFN-β on *IFN-β* transcription through the upregulation of *IRF-7* was also tested by real-time RT-PCR (Fig. 6B): *IFN-β* transcription in NT2-N cells was not significantly affected (1.5-fold increase) by treatment of with either 1,000 or 10,000 IU/ml rIFN-β (result not shown for the highest dose).

A

	CCL-5	IFN-β	OAS-1	IRF-7	STAT-1	RIG-1	TLR-3	NF-κB
1h	**391**	**16**	1	0	0	1	0	0
6h	**13 873**	**30 574**	**898**	**86**	**13**	7.0	2.0	2.0
24h	**19 893**	**787**	**3 372**	**94**	**37**	**107**	**17**	5

B

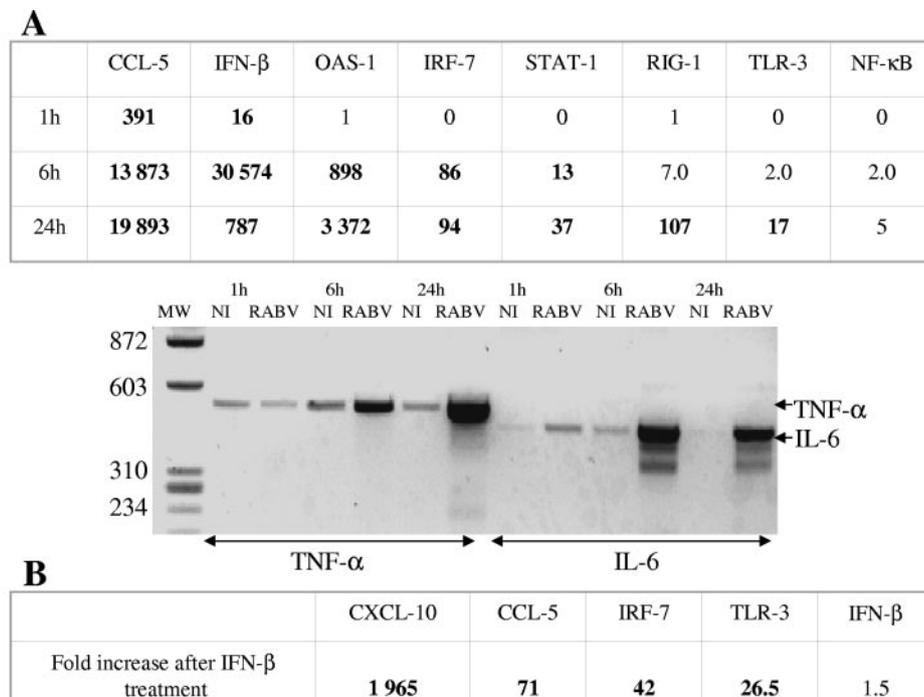|	CXCL-10	CCL-5	IRF-7	TLR-3	IFN-β
Fold increase after IFN-β treatment	**1 965**	**71**	**42**	**26.5**	1.5

FIG. 6. Dynamics of *IFN-β*- and *IFN-β*-controlled mRNA abundances. (A) Kinetics of transcription of a set of genes involved in the innate immune response triggered by RABV infection, as analyzed by real-time PCR or PCR. Results are expressed as multiples of the value for mock-infected cells (defined as 1). Values with relative increases of >10-fold are in bold. The kinetics of the transcriptions of *TNF-α* and *IL-6* during RABV infection were analyzed by RT-PCR. NI, noninfected. (B) Transcriptions of *CXCL-10*, *CCL-5*, *IRF-7*, *TLR-3*, and *IFN-β* after a 24-h treatment of NT2-N cells with IFN-β (1,000 IU/ml). Real-time PCR results are given as multiples of the value for mock-infected cultures (defined as 1).

These observations strongly suggest that NT2-N cells can mount a secondary IFN-β response and therefore that human neurons express IFN-α/βR. However, these cells are not able to increase the expression of *IFN-β* transcripts themselves in the absence of RABV infection.

NT2-N cells can sense synthetic dsRNA but not LPS. NT2-N expressed three molecules, TLR-3 (mRNAs and protein), *PKR* (transcripts only), and *RIG-1* (transcripts only), which can sense dsRNA. Therefore, we analyzed whether these cells can sense the presence of dsRNA in the extracellular compartment. Synthetic dsRNA poly(I:C) (100 μg/ml) was added to the NT2-N cultures, and, after 24 h, the expressions of *IFN-β* and *CCL-5* were analyzed by real-time RT-PCR and those of *TNF-α* and *IL-6* were analyzed by RT-PCR. The response of NT2-N cells to LPS (1 μg/ml) was also tested by real-time RT-PCR (Fig. 7). Treatment with dsRNA led to the upregulation of *IFN-β*, *CCL-5*, *TNF-α*, and *IL-6* transcripts, whereas LPS treatment had no effect (only the real-time PCR data are shown for LPS). Transfection of the cells was not required for an effective sensing of dsRNA (data not shown).

DISCUSSION

We report evidence that human postmitotic neurons (NT2-N cells) express TLR-3 and can mount an innate immune response characterized by the production of IFN-β, chemokines, and inflammatory cytokines in response to RABV and dsRNA [poly(I:C)]. NT2-N can also mount a partial innate immune response to HSV-1 characterized by an increase of

inflammatory cytokine transcripts but not of IFN-β transcripts. Thus, human neurons, even in the absence of astrocytes, oligodendrocytes, and microglia, have the machinery to sense viral infection, and the nature of the innate immune response depends on the nature of the infection.

TLR-3 mRNA has been detected in the human lung, placenta, pancreas, liver, heart, and brain (6, 11, 25, 44, 51). Here, we demonstrate for the first time that human neurons constitutively express TLR-3. Immunocytochemistry confirms the transcriptome analysis findings that *TLR-3* transcripts are present in NT2-N cells. TLR-3 is present mostly in the cytoplasm as cytoplasmic and perinuclear vesicles, as reported for human dendritic cells (44). In addition, there is an immunochemical indication that TLR-3 may also be present at the membrane. RABV infection seems to modify intracytoplasmic distribution of TLR-3, presumably by affecting TLR-3 trafficking, as proposed for TLR-9, which was rapidly redistributed from the reticulum to the lysosomal compartments of human dendritic cells when they had been treated with CpG DNA (39).

TLR-2 mRNAs were present in the NT2-N transcriptome. TLR-2 senses several human viruses, including cytomegalovirus, rubella virus, and HSV-1 and -2 (5, 22). Signaling through TLR-2 results in the activation of NF-κB and the stimulation of inflammatory cytokines, including IL-6. After HSV-1 infection, *IL-6* transcripts were indeed upregulated in NT2-N cells, even though the increase was modest (doubling), suggesting that the increase of *IL-6* transcription may reflect the engage-
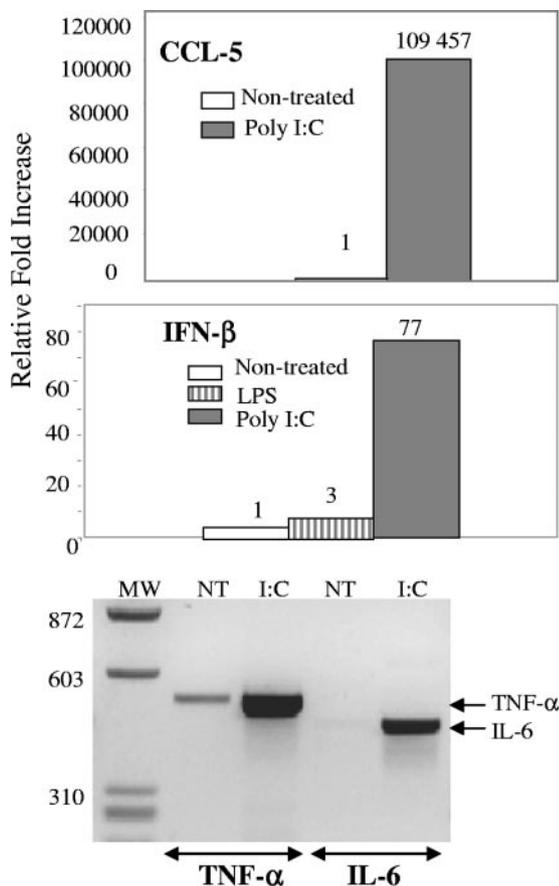
FIG. 7. Synthetic dsRNA but not LPS triggers an innate immune response in NT2-N cells. A 24-h treatment with poly(I:C) (100 μg/ml) triggers the upregulation of *IFN*-β (×77) and CCL-5 (×109,457) transcripts, as determined by real-time PCR, and the upregulation of *TNF*-α and *IL-6* transcripts, as assessed by RT-PCR. LPS has no effect. NT, nontreated.

ment of TLR-2 in HSV-1 detection. Thus, the nature of the TLR expressed by human NT2-N cells supports the idea that human neurons have the machinery to sense RABV and HSV-1 infections. In contrast, NT2-N cells were not reactive to LPS, despite the presence of *TLR-4* in the NT2-N transcriptome. This is consistent with a previous report indicating that the treatment of NT2-N cells with LPS did not result in any detectable TNF-α synthesis, whereas treatment with HTLV-1 Tax protein did (18), and is also consistent with the observation that mouse cortical neurons do not recognize fluorescent LPS (41). Since LPS is recognized by TLR-4 in association with CD14 and MD2, the inability of neurons to sense LPS may result of the default expression of one of these molecules (23).

We provide experimental evidence that human NT2-N cells can sense dsRNA and express genes coding for molecules which can sense dsRNA: *NF-κB*, *RIG-1*, and *PKR* (as transcripts) and TLR-3 (both mRNAs and protein). It is likely that dsRNAs are produced during RABV and HSV-1 infections, and indeed they are byproducts of almost all viral infections. Thus, dsRNA could be the main viral element sensed by human neurons as a trigger of an innate immune response. We do not know which of the group consisting of TLR-3, PKR, and

RIG-1 is involved in this response. Despite the strong expression of TLR-3 in human neurons (Fig. 5B), it is possible that viral components other than dsRNA trigger the innate immune response. Were this the case, neurons would express receptors other than TLR-3 and -2 for sensing viral infection. Further analysis is required to resolve these issues.

The natures and dynamics of the genes recruited by RABV are consistent with the cascade of events of the innate immunity response (illustrated in Fig. 1), with the exception of the positive-feedback loop. Nevertheless, evidence for the involvement of some factors in RABV-induced innate immunity is lacking. For example, the phosphorylation of IRF-3 and the activation of NF-κB cannot be tested by the microarray technique. These points deserve further attention.

Our data clearly indicate that human neurons, in the absence of glia, have the intrinsic machinery to trigger a typical innate immune response, including the two-step interferon response during RABV infection. This is not the case after HSV-1 infection, to which NT2-N cells respond by upregulating a partial innate immune response with an increase of *IL-6* gene transcripts only. The differing patterns of IFN production may reflect the natures of the TLR engaged by the two viruses: TLR-3 is associated with a strong stimulation of IFN-β, inflammatory cytokines, and chemokines in the case of RABV infection, and TLR-2 is associated with inflammatory cytokines only in the case of HSV-1 infection. However, if dsRNA is sensed by human neurons, the innate immune responses to the two viruses should be the same afterwards, because both should produce dsRNA (30). The absence of IFN-β production during HSV-1 infection may be a consequence of the mechanisms expressed by HSV-1 to block IFN production (13, 20, 24, 45, 50).

The decline of *IFN*-β transcription 24 h postinfection in RABV-infected NT2-N cells and the decrease of IFN-β in the supernatants of NT2-N cells infected with RABV for 48 h suggest that RABV may modulate IFN-β production at least in the late steps of infection. Conzelmann and colleagues propose that the P protein of RABV prevents the activation of IRF-3 (8a, 16). In addition, Fu et al., in a companion study in which they compared transcriptomes of mouse NS infected with strains of various pathogenicities, propose that pathogenic RABV controls IFN production in the NS (70a). Nevertheless, RABV seems not to entirely escape the antiviral response it triggers, since an antiviral role, albeit one of limited impact, has been described for IFN-β in RABV infection by Hooper and colleagues (28). RABV infection of human neurons triggers a typical antiviral immune response, with increases of *2′5′OAS-1*, *MxA*, and *GPB* in particular, in addition to a response by IFN-β (43). The antiviral effects of MxA, 2′5′OAS-1, and GTPases have not been shown to limit RABV infection, but such a possibility cannot be excluded. RABV also upregulated the transcription of several chemokines, including *CCL-5*, *-3*, *-4*, and *-20* and *CXCL-11*, *-10*, *-9*, and *-3*, the main function of which is to recruit and attract immune cells (38, 68). Nevertheless, RABV is one of the rare diseases with an almost 100% mortality rate, and it is intriguing that this virus is so neuroinvasive despite causing a strong innate immune response. A similar discrepancy has also been observed for Sindbis virus infection: the most virulent strain of Sindbis virus triggers the strongest production of *CCL-5* mRNAs and *Mx1*

(32). It is possible that the strong early innate immune response observed in this in vitro study corresponds to an only moderate innate immune response in vivo because the response is modulated by pathogenic viral strains, as suggested by Wang et al. (70a). It is also possible that innate immunity is only one component of the subtle balance between the antiviral response of the host and viral virulence.

It is intriguing to note that RABV neuroinvasiveness requires the preservation of neuron integrity to support virus transport through the NS and virus replication (3, 4, 36, 37, 49, 67). In this scheme, neuroprotective factors should favor RABV neuroinvasiveness. Complementary to their inflammatory or antiviral effects, there is increasing evidence that TNF-α, IL-1, IL-6, CCL-5, and even IFN-α/β have neuroprotective functions (7, 14, 27, 29, 46, 55, 57). Thus, it is plausible that a strong innate immune response, as a consequence of the neuroprotective properties of its components, favors RABV neuroinvasiveness. This opens new avenues for the analysis of the possible relationship between neuroinflammation and neuroprotection. The infection of a human neuron-derivative cell line, NT2-N, by neuronotropic viruses, RABV in particular, is a powerful model system to dissect these new mechanisms of neuroprotection.

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