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Transcriptome Signature of Nipah Virus Infected Endothelial Cells

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1. Introduction

The highly pathogenic Nipah virus (NiV) emerged in epidemics in Malaysia in 1998. Regular outbreaks occur since then in Bangladesh and India with the high mortality rate reaching up to 90%. During the first emergence in Malaysia, the only way to contain the outbreak was culling of more than one million pigs leading to major economic issues, estimated at over US$ 100 million (Lee, 2007). Thus, NiV is considered as a potential agent of bioterrorism and is designated as priority pathogens in the National Institute of Allergy and Infectious Diseases (NAID) Biodefense Research Agenda. Neither treatment nor vaccines are available against NiV infection, limiting thus experimentation with live virus to Biosafety level 4 (BSL4) laboratories, which require the highest level of precaution.
Nipah virus infection is often associated to the development of the wide spread vasculitis but molecular basis of its pathogenicity is still largely unknown. To gain insight in the pathogenesis of this highly lethal virus we have performed analysis of virus-induced early transcriptome changes in primary endothelial cells, which are first targets of Nipah infection in humans.

1.1 The virus

Together with the closely related Hendra virus (HeV) that appeared in Australia in 1994, NiV has been classified in the new genus called Henipavirus, in the Paramyxoviridae family. Placed in the order of the Mononegavirales, this family has nonsegmented single stranded negative-sense RNA genome (Lamb & Parks 2007). Henipavirus encodes 6 structural proteins: the nucleocapsid N, phosphoprotein P, the matrix protein M, fusion F, attachment G, and the large polymerase L. The P gene also codes for non-structural protein through two different strategies. First, by mRNA editing, pseudotemplated guanosine residues could be inserted causing a frame shift of either 1 or 2 nucleotides leading to the production of the proteins V and W. The C protein is produced through the initiation of translation of P mRNA at an alternative start codon 20 nucleotides downstream in the +1 ORF (Wang et al., 2001). Because of its short length, the C protein can be produced through P, V and W mRNAs (Fontana et al., 2008).

1.2 Epidemiology

Numerous studies have demonstrated that the natural hosts of NiV are flying foxes in the genera *Pteropus* and *Eidolon* in South-East Asia as well as in Madagascar (Iehlé et al., 2007) and Ghana (Drexler et al., 2009). The emergence of NiV as zoonosis could be due to the fact that large areas of South East Asia have recently been subject to deforestation. Consequently, breeding territories of giant bats have been found in close proximity to people habitation, which has facilitated contact with domesticated animals as well as with humans. Since its emergence in Malaysia in 1998, NiV was shown to be different from the other members of its family by its capacity to cause the most important zoonosis ever observed within Paramyxoviridae. Indeed, during this first outbreak, the virus infected humans, pigs, cats, dogs and horses (Maisner et al., 2009). Among infected people, about 90% were working in pig farms. Serological analysis revealed that pigs were responsible for the transmission of Nipah virus to humans. Therefore, in order to contain this first occurrence, more than 1 million pigs were culled. Although it seems that Nipah outbreaks have been stopped in Malaysia, the virus continues to cause regular outbreaks from 2001 up to nowdays in India and Bangladesh. However, pigs were not involved in those outbreaks, and virus seemed to be transmitted directly form its natural reservoir fruit bats, to humans. Fruit bats from Malaysia, Cambodia, Bangladesh and Thailand were tested and the studies revealed the existence of new strains of NiV (Halpin & Mungall 2007). Even the virus can pass via an intermediate host like pigs, viral transmission occurs during last few years from bats to humans through palm juice (Luby et al., 2006) and has been responsible for reappearance of NiV in 2010 (17 deaths) and 2011 (35 deaths) increasing the total number of NiV outbreaks to 13 since its first appearance (Nahar et al., 2010)(Salah et al., 2011). Finally, human to human transmission has been documented in more than half of the outbreaks (Gurley et al., 2007, Luby et al., 2009).

1.3 NiV tropism

NiV can naturally infect a large panel of mammals suggesting the high conservation of its receptor among them (Eaton et al., 2006). In addition, the glycoproteins G of the Henipavirus show a tropism for a number of different cell types including neural, endothelial, muscular and epithelial cells (Bossart et al., 2002). Ephrin B2 (EFN B2) has been demonstrated as the receptor for both NiV and HeV. Indeed, this highly conserved protein is expressed at the surface of all permissive cell lines. Moreover, the transfection of cells with the gene coding for EFN B2 makes them permissive to the infection (Negrete et al., 2005). EFN B2 is essential to vasculogenesis and neuronal development. This transmembrane protein of 330 aa is expressed by numerous cells, but more particularly at the surface of epithelial, endothelial, smooth muscles and neuronal cells, that show the highest level of viral antigens during infection in patients (Lee, 2007). Finally, despite the high affinity of NiV for EFN B2, its expression at the surface of cells is not always sufficient for the virus entry, suggesting the existence of an additional receptor or intracellular factor necessary for viral replication (Yoneda et al., 2006).

The second entry receptor for NiV and HeV has been identified: Ephrin B3 (EFN B3), with the affinity for NiV 10 times lower than EFN B2 (Negrete et al., 2006). EFN B3 is a transmembrane protein of 340 aa. At the position 121 and 122 of EFN B3 and B2, 2aa appear essential for the virus entry. In contrast to EFN B2, EFN B3 is more expressed at the level of the brainstem, which could be linked with the severity of the neuron dysfunctions during the NiV encephalitis (Negrete et al., 2007).

1.4 The pathology in humans

After incubation period which varies from 4 to 60 days, NiV infection starts similarly to flu. In the large majority of the cases patients present fever, whereas 2/3 of them develop headache, leading frequently to severe acute encephalitis with loss of consciousness. Some of patients develop in addition respiratory symptoms. Death occurs in 40 to 90% within an average time of 10 days post fever, due to the severity of the cerebral damages (Lee, 2007).
The pathology is characterized by a systemic vasculitis with syncytia formation of microvascular endothelial and epithelial cells (Fig. 1). Perivascular cuffing is generally observed. Despite the fact that the virus infects all organs, the microvascularization of central nervous system shows the most severe damages.

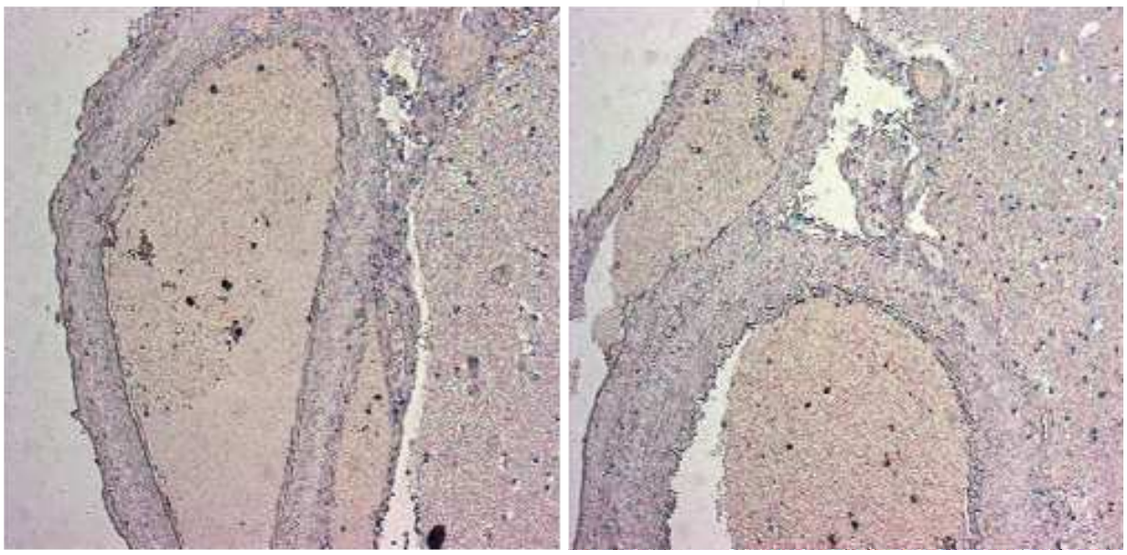

Fig. 1. Photos of hematoxylin staining of cerebral cortex of patients infected with NiV during the first outbreak in Malaysia, showing widespread vasculitis (personal data)

Patients show wide lymphoid necrosis associated to giant multinucleated cells that could be related to the presence of the NiV in this tissue. Virus may propagate initially within the lymphoid tissue, leading to the infection of the endothelial cells, recognized as the first primary targets of NiV. Those cells allow the second cycle of replication of the virus and the viremia.
NiV infection is characterized by the formation of syncytia leading to the endothelial damages, which are thought to be the cause of thrombosis, inflammation, ischemia and finally necrosis. Resulting vascular infarctions and infiltrates lead to extravascular infection and parenchymatous invasion. The invasion of the central nervous system is generally followed by the lethal encephalitis.
Patients who have survived the NiV infection showed severe weakness sometime persisting for several months, and often complicated by neurological and/or motor dysfunctions (Sejvar et al., 2007). Those symptoms appear as a direct consequence of the acute encephalitis. Indeed, those patients develop atrophy of the cerebellum, brainstem lesions, cortical nervous transmission abnormalities and are particularly affected in the white matter (Ng et al., 2004). In Malaysia, about 7,5% of patients who survived the encephalitis had relapsed during the year following their infection without any reexposure to virus. In

addition, NiV can cause apparently asymptomatic infection leading to the late onset encephalitis several months to a year after infection (Tan et al., 2002). This fact suggests that the virus can infect more people than those showing clinical symptoms and may stay in latent stage until reactivation under the influence of some still unknown factors.

1.5 Vaccines and treatments

Several studies have been focused on the development of anti-NiV vaccines. The first study has shown that hamsters, vaccinated with vaccinia virus expressing either NiV F or G, were completely protected against NiV. Moreover, this group demonstrated that the naïve animals were also protected by passive transfer of hyperimmune serum prior to challenge (Guillaume et al., 2004). An important advance was next the development of a recombinant vaccine protecting pigs against NiV challenge (Weingartl et al., 2006). The Canarypox virus expressing NiV glycoproteins was shown to be very efficient in pigs and may have a real socio-economic interest in the case of new NiV outbreaks. Recently, one group showed induction of neutralizing antibodies to Henipavirus using an Alphavirus based vaccine (Defang et al., 2010). However, the study has been performed in mice which are not sensitive to NiV infection (Wong et al., 2003), preventing them from testing the efficiency of the vaccination.

Monoclonal antibodies against NiV glycoproteins were shown to protect 50% of infected hamsters even when treatments started 24 h post infection (Guillaume et al., 2006) and anti-NiV F monoclonal antibodies protected hamsters against Hendra virus infection as well (Guillaume et al., 2009). In addition, neutralizing human monoclonal antibody protected ferrets from NiV infection, when given 10 h after oronasal administration of the virus (Bossart et al., 2009).

Treatment of NiV infection was tested using some of known anti-viral chemicals: ribavirin (Chong et al., 2001), chloroquine (Pallister et al., 2009), gliotoxin, gentian violet and brilliant green (Aljofan et al., 2009). Most of those products showed an effect either *in vitro* or *in vivo* but with too low efficiency to consider them as a good treatment for infected patients, even if they were used combined (Freiberg et al., 2010). Finally, anti-fusion peptides were designed that specifically target the entry of Henipavirus (Porotto et al., 2010). To improve the efficiency of this potential treatment, this group has added a cholesterol tag, highly increasing the anti-viral efficiency and allowing peptides to reach brain and limit viral entry into cerebral cells, giving thus very promising results both *in vitro* and *in vivo* in hamsters (Porotto et al., 2010). This new anti-viral approach needs now to be tested in a primate model to consider its potential utilization in humans.

2. Global gene expression analysis of NiV infected endothelial cells, using microarrays

Profound changes are occurring in host cells during viral infections. These pathogen-induced changes are often accompanied by marked changes in gene expression and could be followed through the analysis of the specific RNA fingerprint related to each virus (Glass et al., 2003), (Jenner et al., 2005). For this purpose, microarrays present the essential tool to study global changes in gene expression and better understand which cellular mechanisms are modulated during the viral replication cycle. The aim of this study was to obtain a global overview of NiV effect on endothelial cells, in order to open new perspectives In treatment of this lethal infection.

Very little is known on pathogenesis of NiV infection. To obtain the global insight in different host cell changes during the infection, we have performed gene expression analysis using microarrays. *In vivo*, primary targets of this virus are endothelial cells, smooth muscles and neurons. The infection of microvascular endothelial cells leads to a generalized vasculitis, which is the common symptom diagnosed among all infected animals and humans. This vasculitis usually induces the acute encephalitis that is observed in severe NiV infection. Therefore, primary human endothelial cells were chosen as the most relevant host cell type to analyze the effect of NiV infection on the host cell gene expression.

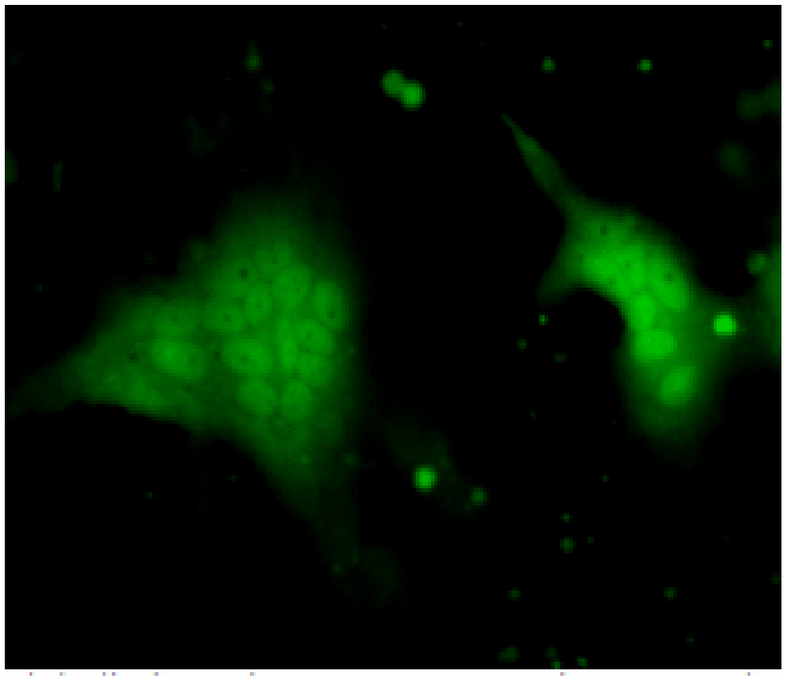

Fig. 2. HUVEC infected with the NiV recombinant strain expressing EGFP (MOI=1) for 24h and presenting a large syncytia, observed under the fluorescent microscope.

2.1 HUVEC culture and NiV infection

We have, thus, analyzed the effect of NiV infection in primary human umbilical vein endothelial cells (HUVEC). These cells are highly permissive to NiV infection and develop large syncytia rapidly after infection, as shown when recombinant NiV expressing the fluorescent green protein EGFP (Yoneda et al., 2006) is used for infection (Fig. 2). Primary HUVEC cells were isolated from umbilical cords of 6 donors (Jaffe et al., 1973). Cells were then transferred in a 75ml flask, precoated with gelatin 0,2% in PBS for 30 min and washed. The following day, cells were trypsinated to eliminate any dead or residual blood cells, and pooled by 2 sets of 3 donors and put in new flasks in order to cover 50% of the surface. After one week of culture, cells were submitted to 16 hours of serum privation just before their infection.

The infection was performed using wild type NiV (isolate UM-MC1, Gene accession N°AY029767) at MOI 1, in 2 sets of 3 different donors, in BSL4 Laboratory Jean Mérieux in Lyon, France.

2.2 Microarray experiments

Early changes associated to initial stages of NiV infection were analyzed by microarray approach (Fig. 3). Total RNAs were extracted from infected cells at 8h post infection and from uninfected cells (mock) cultured in the same conditions. Quality of total RNA was checked on Agilent bioanalyzer 2100. Amplified and biotin-labeled RNAs were obtained from 2µg of total RNA, using the Ambion message Amp kit version II. Different quantities of positive RNA controls (spikes) were added during the first step of reverse transcription of total RNAs. Spikes correspond to 6 bacterial RNAs used to control sensitivity, quality of hybridization and data normalization. Hybridization was performed on Codelink human whole genome bioarray (http://www.codelink bioarrays.com/) that is a 3-D aqueous gel matrix slide surface with 30-base oligonucleotide probes. This 3-D gel matrix provides an aqueous environment that allows an optimal interaction between probe and target and results to higher probe specificity and array sensitivity. Codelink uses a single color system (1 array/sample).

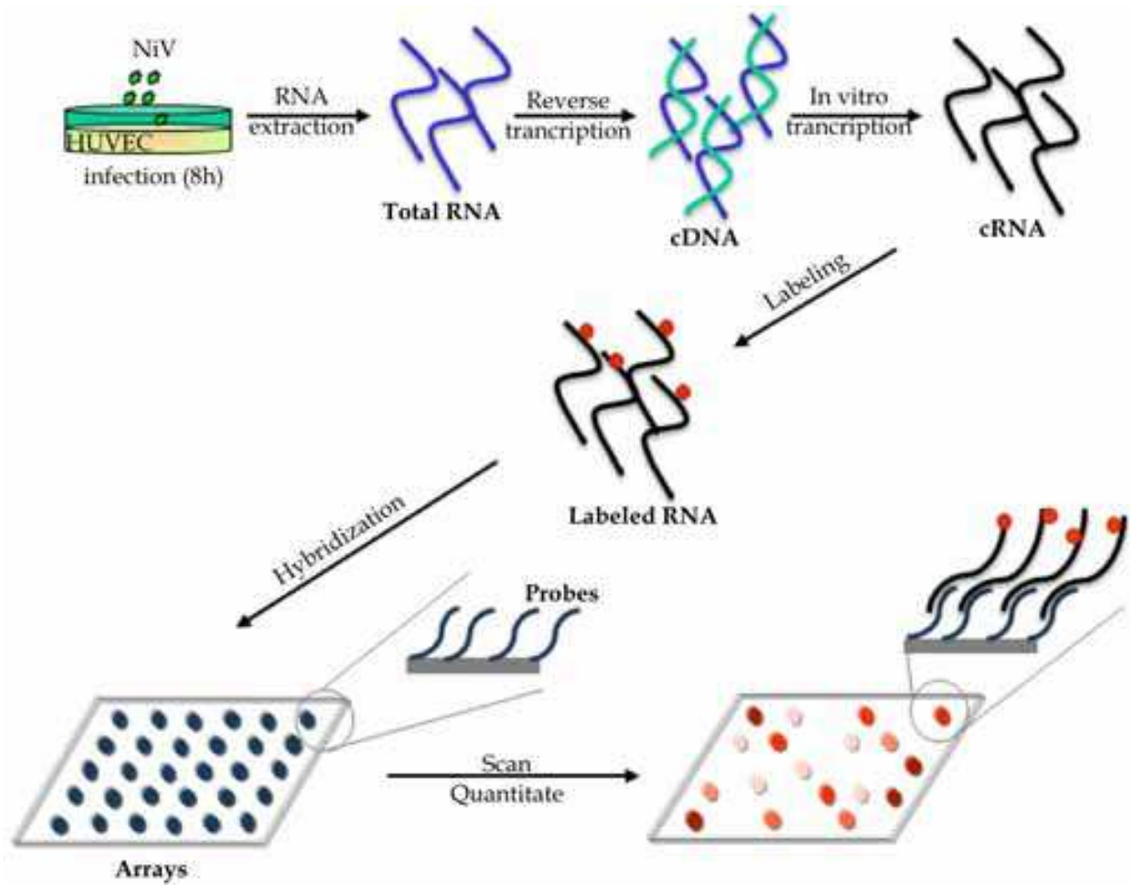

Fig. 3. Representation of the different steps necessary for the microarray analysis, starting from NiV infection of HUVEC cultures up to the analysis of microarrays.

Codelink human whole genome bioarray comprises approximately 55,000 30-mer probes on a single array based on the NCBI/Unigene database that permits the expression analysis of 57,347 transcripts and ESTs. In addition to these 55,000 probes, Codelink human whole

genome bioarrays also contain one set of 100 housekeeping genes, 108 positive controls and 384 negative controls (bacterial genes). Hybridization, wash and revelation were performed using Codelink Expression Assay reagent kits. Then, chips were scanned using an Axon Genepix 4000B Scanner. Data extraction and raw data normalization were performed using the CodeLink Gene Expression Analysis v4.0 software. Normalization was performed by the global method. The threshold was calculated using the normalized signal intensity of the negative controls supplemented by 3 times the standard deviation. Spots with signal intensity below this threshold are referred to as "absent". Finally, data are converted to the excel format and data analysis is performed by using the Gene Spring v7.0 software from Agilent.

2.3 Microarray data analysis

The effect of NiV on the modulation of the genes expression was determined by permutation analysis and we considered as pertinent a minimal fold change (FC) of 1,3. Among the 55,000 targeted genes, 1076 genes were found to be differentially expressed in NiV-infected cells in comparison to non infected cells, including 807 up-regulated genes ($1.3 \leq FC \leq 23$) and 269 down-regulated ($-46 \leq FC \leq -1.3$) genes. These 807 up-regulated genes were then classified according to their Gene Ontology (GO) biological processes and their GO molecular functions. This system of clustering takes into account not only the number of genes but also the importance of the modulations in each function. Most of the cellular functions were modified after NiV infection (Fig. 4A). This could be explained by the modulation of some key genes involved in the large majority of the known functions. The most importantly modulated functions were those belonging to "Immune Response" with 37 differentially regulated genes (Table 1A) and to "Organism Abnormalities and Injuries" (22 genes), two functions that are usually altered in case of productive viral infection. Surprisingly, this analysis also revealed changes in the "neurological diseases" function (15 genes) and "nervous dysfunctions" (5 genes). This result could be correlated with the strong involvement of the endothelial cell-induced inflammatory reaction in the development of the encephalitis, as described in the introduction.

To refine the significance of these up-regulated genes, we next investigated the biological functions and interactions of these genes using Ingenuity Pathway Analysis (IPA) software. IPA allows genes that are differentially expressed to be placed in a physiological and biochemical context by grouping them according to canonical pathway and biological network with a statistical probability of validity, based on number of genes being differentially expressed in the respective pathway. This IPA analysis allowed us to identify that the most significantly modulated canonical pathway is the "interferon signaling" pathway ($p=0,01$) (Fig. 4 B). The majority of the top 15 up-regulated genes are related to the Interferon pathway (Table 1B). The involvement of the Interferon pathway has been proposed in the development of the other types of vasculitis, including the post-operative vasculitis (Abe et al., 2008). Four other canonical pathways were significantly found modified during the infection by NiV: "Antigen presentation", "Integrin signaling", "Protein Ubiquitination" and "Nicotinate and Nicotinamide Metabolism" pathways. Finally, IPA allowed us to demonstrate the existence of network of genes involved in the pathway of Gene expression, Cell Death, Connective tissue disorders (Fig. 5). Some of these gene, like TLR3 (Shaw et al., 2005) and CXCL10 (Lo et al., 2010), have been already shown to be associated to NiV infection, while the role of other genes rests to be demonstrated.

A

Gene	Accession number	Fold change
MX1	AF135187	23,253
IFI44L	NM_006820	14,528
CXCL10	NM_001565	10,93
OAS1	AU076579	8,6234
IFI44	NM_006417	5,7188
MX2	NM_002463	5,179
CCL8	NM_005623	4,8824
PLSCR1	AW439730	3,9274
CXCL11	NM_005409	3,538
TAP1	NM_000593	2,9255
TNFSF13B	AI446030	2,8342
STAT1	AK022231	2,5021
PSMB9	NM_002800	2,494
SP140	NM_007237	2,4399
IFI6	NM_002038	2,1447
IRF-1	NM_002198	1,9537
ZC3HAV1	BX108858	1,8427
STAT2	NM_005419	1,803
SECTM1	AA601122	1,7953
MYD88	NM_002468	1,7122
ISG20	NM_002201	1,6514
TRIM22	NM_006074	1,6078
PECAM1	BG739826	1,5874
HSPD1	NM_002156	1,5844
PSMB8	NM_004159	1,5805
TLR3	NM_003265	1,4589
IL15RA	NM_002189	1,4529
CCRL1	NM_016557	1,4023
HLA-E	NM_005516	1,383
CD47	NM_001777	1,3585
REV3L	NM_002912	-1,3367
MAPKAPK2	R97920	-1,36484
ABCA1	NM_005502	-1,40317
IL17RB	NM_018725	-1,55537
FKBP1A	BQ004596	-1,56654
CD1B	NM_001764	-2,1301
NRP1	AI285044	-2,24691

B

Gene	Accession number	Fold change
MX1	AF135187	23,253
IFIT1	NM_001548	19,764
IFIT3	NM_001549	10,793
OAS1	AU076579	8,6234
IFI35	NM_005533	3,3087
TAP1	NM_000593	2,9255
STAT1	AK022231	2,5021
IRF1	NM_002198	1,9537
STAT2	NM_005419	1,803
ISGF3G	NM_006084	1,7891
PSMB8	NM_004159	1,5805

C

CEB1	NM_016323	5,3687
FLJ20637	NM_017912	5,1178
BBAP	NM_138287	4,8868
ISG43	NM_017414	2,9878
TAP1	NM_000593	2,9255
LMP2	NM_002800	2,4940
RIG-B	NM_004223	2,4861
RO52	NM_003141	2,1784
FBG2	NM_018438	1,8813
IFP1	NM_021616	1,8357
IRF9;ISGF3	NM_006084	1,7891
PAD1	NM_005805	1,6306
RNF94	NM_006074	1,6078
MULE	NM_031407	1,5970
LMP7	NM_004159	1,5805
UNPH4	NM_006313	1,5614
EFP	NM_005082	1,5047
TRIM19	NM_033238	1,4574
TRIM5alpha	NM_033034	1,4573
FAT10	NM_006398	1,4544
USP34	BX099597	1,4495
NACSIN	NM_015252	1,4330
PSMA7L	NM_144662	1,4273
ARIH1	AI656728	1,3908
UBE2D3	BQ960542	1,3694
HRCA1	NM_007218	1,3652
APC4	NM_013367	1,3563

Table 1. Genes differentially expressed during NiV infection in the Immune Response (A), Interferon pathway (B), protein ubiquitination pathway (C).

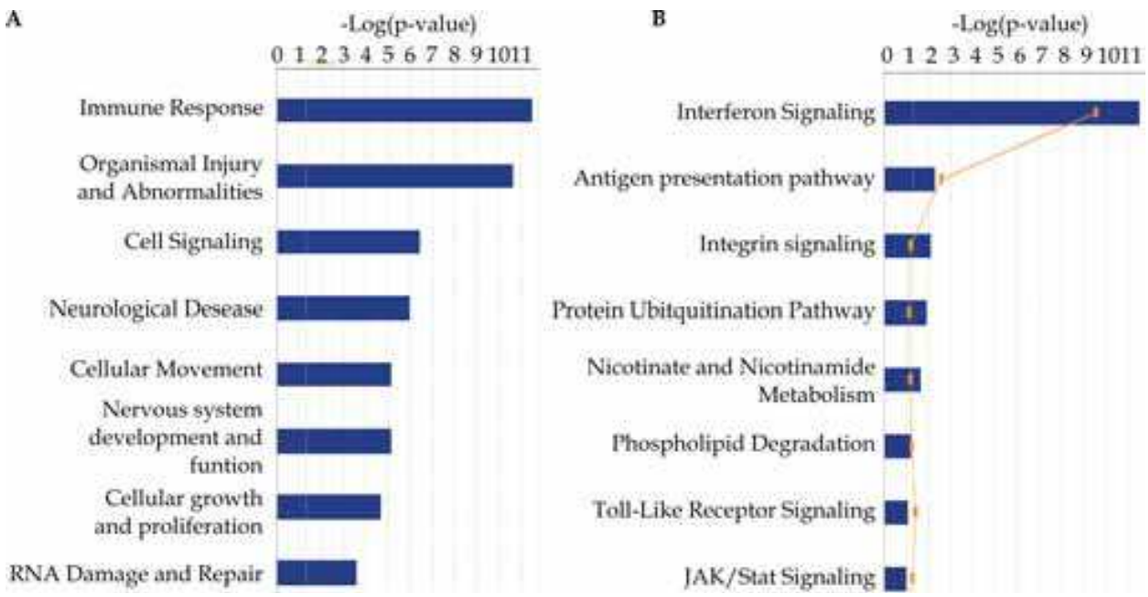

Fig. 4. Impact of NiV infection on biological functions (A) and canonical pathways (B), determined using Ingenuity Pathway Analysis.

Score	Focus Genes	Top Functions
56	35	Gene Expression, Cell Death, Connective Tissue Disorders
24	21	Organismal Injury and Abnormalities, Cellular Movement, Hematological System Development and Function
16	16	Cellular Movement, Hematological System Development and Function, Immune Response
16	16	Immune Response, Cell-To-Cell Signaling and Interaction, Hematological System Development and Function
16	16	Cell Death, Carbohydrate Metabolism, Cellular Assembly and Organization

Table 2. Putative Networks with high score, identified by Ingenuity Pathway Analysis

In addition, this IP analysis revealed that the 2 top putative networks with high score (> 20) were strongly associated with the "Connective Tissue Disorders" and the "Hematological System Development and Function" (Table 2). As microvascular basal lamina plays a critical role in brain injury (Wang & Shuaib, 2007), the loss of basal lamina components may reflect the degradation of proteins by proteolitic enzymes.

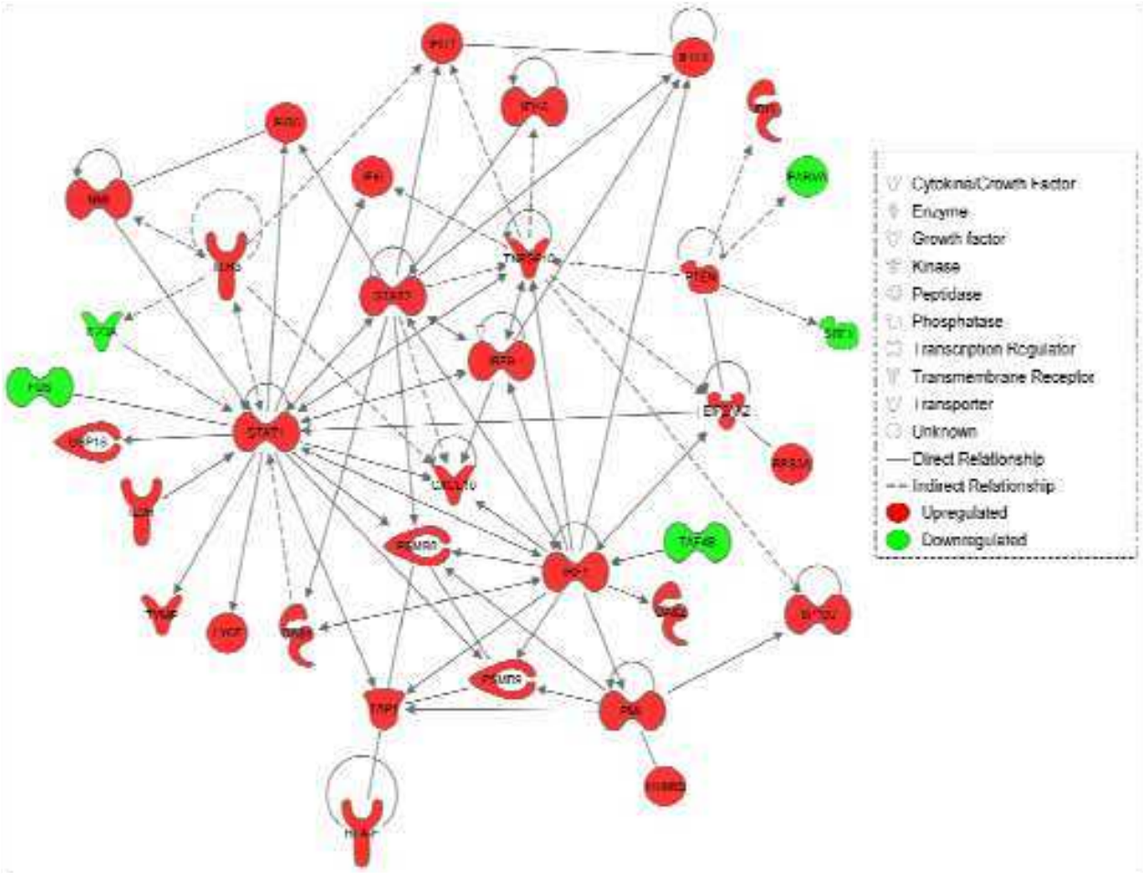

Fig. 5. Gene network identified in NiV infected HUVEC, compared to mock infected controls, reveals involvement of different genes within the pathways of Gene expression, Cell Death and Connective tissue disorders.

2.4 Validation of genes by quantitative real time PCR

To validate data obtained by microarray, we compared mRNA levels of several highest upregulated genes involved in the Immune response, between NiV infected and uninfected cells. These genes included Mx1, OAS1, CXCL10, CXCL11, PSMB9 (also known as LMP2) and RIGB. Total RNA were extracted 8 hours post-infection. Reverse transcriptions were performed on 0,5 µg of total RNA using the iScript cDNA synthesis kit (Bio-Rad) and run in Biometra® T-GRADIENT PCR devise. Obtained cDNAs were diluted 1/10. Quantitative PCR was performed using Platinum® SYBR® Green qPCR SuperMix-UDG with ROX kit (Invitrogen™). qPCR was run on the ABI 7000 PCR system (Applied biosystems) using the following protocol: 95°C 5', and 40 cycles of 95°C 15'', 60°C 1', followed by a melting curve up to 95°C at 0.8°C intervals. All samples were run in duplicate and results were analyzed using ABI Prism 7000 SDS software available in the genetic analysis platform (IFR128 BioSciences Lyon-Gerland). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene for viral mRNA quantification and normalization. GAPDH and standard references for the corresponding genes were included in each run to standardize results in respect to RNA integrity, loaded quantity and inter-PCR variations. Primers used were design using Beacon 7.0 software, and validated for their efficacy close to 100%: RiGB

for: ATCATCAGCAGTGAGAAC, RiGB rev: GAACTCTTCGGCATTCTT, LMP2 for: GGTCAGGTATATGGAACC, LMP2 rev: CATTGCCCAAGATGACTC, GAPDH for: CACCCACTCCTCCACCTTTGAC, GAPDH rev: GTCCACCACCCTGTTGCTGTAG. The relative expression represents the ratio of the number of copy of mRNA of interest versus mRNA of GAPDH. All calculations were done using the $2^{\Delta\Delta CT}$ model of (Pfaffl, 2001) and experiments were performed according to the MIQE guideline (Bustin et al., 2009).

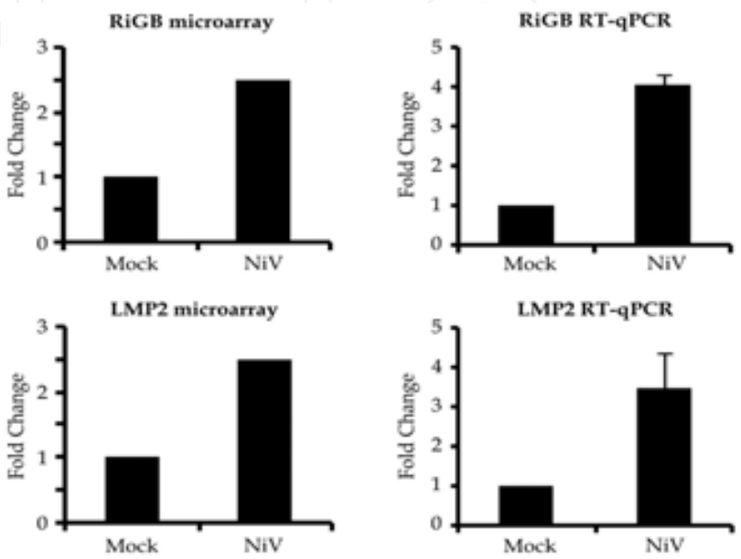

Fig. 6. Example of genes used for the validation of the microarray data. Results obtained from the microarray are shown on the left, whereas RT-qPCR data for the same gene are shown on the right.

2.5 Focus on some genes of interest

Among the cellular pathways activated during the NiV infection, we have particularly focused our attention to the interferon related genes. We have observed that similarly to the other Paramyxoviruses, NiV strongly activates the immune response through the canonical interferon signaling pathway. An over expression of some interferon related genes is known to lead to the activation of several genes related to the proteasome and the ubiquitination pathway. Those genes are involved in loading and expression of the CMH class 1 at the cell surface. In fact, any imbalances in this system can lead to a strong deregulation, resulting in inflammation that could not be controlled by host homeostatic mecahanisms (example: lupus erythematous, (Baechler et al., 2004). TAP1/2 and LMP2 (PSMB9) are the major proteins involved in this system, and both were shown to be up-regulated during NiV infection of endothelial cells (Table 1). The expression of those proteins is regulated by Interferon related proteins called Signal Transducer and Activator of Transcription 1α (STAT1α) and Interferon Regulatory Factor 1 (IRF1) (Chatterjee-Kishore et al., 1998). In normal conditions, TAP1 and 2 are expressed at a basal level in cells, whereas LMP2 is not found (Wright et al., 1995). Our results show that NiV infection increases TAP1 expression without any changes in TAP2. Moreover, LMP2 was also induced. An imbalance in the expression of those proteins that are the major components of the immunoproteasome, are in certain cases responsible for important phenomena of autoimmunity leading to severe

damages on the endothelium, including systemic vasculitis in case of Lupus (Zimmer et al., 1998) and may be therefore involved in the pathogenesis of NiV induced vasculitis.
In addition, our results revealed NiV-induced up regulation of HERC5 (FC=5,37), which belongs to the E3 ubiquitin ligases family. This protein has been shown to be tightly controlled under inflammatory conditions in endothelial cells (Kroismayr et al., 2004). The critical role of Tumor necrosis factor α, Interleukin 1β and NF-KB was suggested in the regulation of this protein. Although we have observed an over expression of TNFSF13b in the NiV infected HUVEC, modulation of IL1β or NF-KB was not found, suggesting another cascade of NiV-induced activation of HERC5.
Virus infection is known to induce a specific chemokine production in infected cells. This chemokine response is often related to the detection of viral genomes by Toll like receptor (TLR) system. Depending on the combination of TLRs involved in this mechanism it will lead to a specific signature of expression. For example, the closely related Measles virus induces several chemokines CCL2, CCL3, CCL4, CCL5 and CXCL10 (Glass et al., 2003). Only CXCL10, CXCL11 and CCL8 were induced by NiV infection in HUVEC (Table 1), suggesting a high capacity of NiV to provoke an imbalance of cell signaling, leading to a miss regulation of inflammation. These results are in accord with demonstrated changes in cytokine production by endothelial cells (Lo et al., 2010). The strong involvement of Interferon related genes in the vasculitis has been described before, but the induction of the monocyte chemoattractant CCL8 remains unclear in the context of a viral infection. Indeed, very few viruses are inducing this protein (Glass et al., 2003). Nevertheless, CCL8 has been shown to be involved in many inflammatory diseases including rheumatoid arthritis (Galligan et al., 2007), (Ockinger et al., 2010) and Graft versus host diseases (Bouazzaoui et al., 2009). The functional importance of CC chemokine ligand genes has been demonstrated in experimental autoimmune encephalomyelitis and multiple sclerosis (Mahad et al., 2004), (Savarin-Vuaillat & Ransohoff 2007). CCL8 is overexpressed by astrocytes and microglia leading to the over recruitment of monocytes and macrophages to the lesions (Vyshkina et al., 2008). This result suggests the importance of regulation of CCL8 either at the genomic level or within the chemokine network, when the virus reaches the brain.
Furthermore, within genes involved in the cellular movement function NiV induced the expression of ADAM 12 (FC=2,38). This protein is a metalloprotease proposed to function as a regulator of fusion of several cell types, including trophoblast and myoblast (Huppertz et al., 2006). This protein also modulates the cell fusion in giant cell tumors of long bones (Meng et al., 2005), by inducing actin cytoskeleton reorganization. This reorganization could be associated to the remodeling of actin induced by NiV binding to its receptor and consequent EFNB2 signaling. In addition to the capacity of ADAM12 to reorganize the extracellular matrix, its over expression in endothelial cells could be related not only to the syncytia formation but also to microvascular basal lamina damages. This phenomenon causes dismantlement of the endothelial wall structure (Wang & Shuaib, 2007). Such microvascular permeability in the brain could compromise the microcirculation by increasing the risk of ischemia and the exposure of this compartment to the immune system, leading thus to an important vascular and perivascular inflammation.

3. Conclusions

NiV is a highly lethal zoonotic pathogen that can cause important socio-economical and health problems. This virus induces a generalized vasculitis leading to the disruption of the

endothelial microvascular tissue in brain and inducing severe damages in the CNS. Micorarray analysis of NiV infected primary endothelial cells allowed us to obtain a global overview of the host cell responses to NiV early during the infection. This global approach revealed that NiV infection has an important impact in several pathways and functions that are directly related to the pathogenesis observed in patients and animals. The analysis revealed a high induction of the immune response through the important modulation of genes in the Interferon signaling pathway, Antigen presentation pathway and the Protein ubiquitination pathway. We focused our analysis on several highly induced genes which could be involved in the control of the vascular inflammation and disruption of endothelium, allowing the passage of the virus in the organs. The early NiV infection of endothelial cells importantly upregulated the chemokines TNFSF13B, CXCL10, CXCL11 and CCL8 that are involved in many processes of autoimmune diseases as well as proteins belonging to the ubiquitination pathway. More precisely, TAP1 and LMP2 were overexpressed during the infection. NiV-induced sustained inflammatory conditions and modified regulation of the immunoproteasome expression could lead to an imbalance of the MHC class 1 exposure at the surface of cells, inducing haemostatic disturbance during NiV infection. This study presents the first comprehensive analysis of global host transcriptional response to NiV infection. Obtained results shed new light to early stage of NiV pathogenesis and should help in understanding the host response to this virus and open perspectives for design of treatment for this emerging lethal infectious disease.

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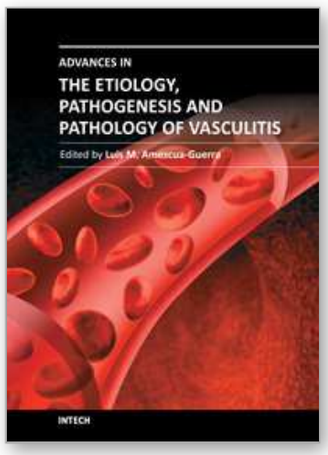

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This book represents the culmination of the efforts of a group of outstanding experts in vasculitis from all over the world, who have endeavored to devote their work to this book by keeping both the text and the accompanying figures and tables lucid and memorable. Here, you will find an amalgam between evidence-based medicine to one based on eminence, through an exciting combination of original contributions, structured reviews, overviews, state-of the-art articles, and even the proposal of novel pathogenetic models of disease. The book contains contributions on the etiology and pathology of vasculitis, the potential role of endothelial cells and cytokines in vascular damage and repair as well as summaries of the latest information on several primary and secondary vasculitis syndromes. It also covers selected topics such as organ-specific vasculitic involvement and quality of life issues in vasculitis. The editor and each of the authors invite you to share this journey through one of the most exciting fields of the medicine, the world of Vasculitis.

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