ARTICULO DE REVISION

Antiviral responses to dengue virus

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ABSTRACT

Dengue virus (DENV) produces a wide range of illness, going from asymptoBmatic infections to hemorrhagic disease. This mosquito-borne flavivirus causes about 50 million infections annually (mainly in the tropics), most them resulting in a febrile illness. Dengue hemorrhagic fever (potentially fatal vascular leakage syndrome), appears less frequently. The innate immune response of the host plays an important role during the initial stages of infection. Severe disease is associated with high viremias, immune enhancement of sequential infections and exacerbated inflammatory response. DENV is sensed in mammalian cells by endosomal and cytoplasmic receptors and stimulates the host innate immune response, in particular the type-1 interferon (IFN α/β) response which acts on target cells by stimulating the JAK/STAT signaling network. This results in activation of genes that lead the infected cells toward an antiviral response. Genomic technology allowed the identification of human genes induced in response to DENV. The results define common antiviral and pro-inflammatory responses composed mainly of IFN α/β induced genes, which likely participate in the regulation of the immune response and induce vascular leakage during the acute phase of the disease. DENV can also circumvent the IFN α/β response of the host. Apparently, non-structural proteins of DENV weaken IFN α/β signaling, causing reduced response in activation of gene expression. Increased virus uptake, weakening of the host cell defense, and unrestrained inflammatory response likely predispose patients to develop severe illness. The identification of antiviral response signature genes and the discovery of crucial virus-host interactions lead to a better understanding of the molecular mechanisms of viral pathogenesis and will serve to improve diagnosis and therapy.

Key words: Dengue Virus, interferon, IFN α/β, JAK/STAT signaling

RESUMEN

Respuestas antivirales al virus dengue

El virus Dengue (VDEN) produce una gran variedad de enfermedades que van desde infecciones asintomáticas hasta enfermedad hemorrágica. Este flavivirus, portado por mosquitos, causa unas 50 millones de infecciones anualmente (principalmente en los trópicos), resultando la mayoría en enfermedad febril. El Dengue hemorrágico (síndrome de derrame vascular potencialmente fatal) es menos frecuente. La respuesta inmune innata del hospedador juega un papel importante durante la fase inicial de la infección. La enfermedad severa está asociada con viremia alta, incremento de inmune por infecciones secuenciales y respuesta inflamatoria exacerbada. El VDEN es detectado en células de mamíferos mediante receptores endosomlaes y citoplasmáticos y estimula la respuesta inmune innata del hospedador, en particular la respuesta de interferón tipo 1 (IFN α/β) que actúa en sus células blanco estimulando la red de señalización de JAK/STAT. Esto resulta en la activación de genes que determinan una respuesta antiviral de las células infectadas. La tecnología genómica ha permitido la identificación de los genes humanos inducidos en respuesta al VDEN. Los resultados definen respuestas antivirales y pro-inflamatorias comunes compuestas principalmente por genes inducidos por IFN α/β, que probablemente participan en la respuesta inmune e inducen derrame vascular durante la etapa aguda de la enfermedad. El VDEN puede también esquivar la respuesta inmune del hospedador. Aparentemente, las proteínas no estructurales del VDEN debilitan la señalización del IFN α/β, reduciendo la respuesta de activación de expresión genética. Incremento en la entrada de virus, debilitamiento de la defensa celular, y respuesta inflamatoria incrementada, predisponen al desarrollo de enfermedad severa. La identificación de los genes involucrados en la respuesta antiviral y el descubrimiento de interacciones cruciales virus-hospedador han servido para entender mejor los mecanismos moleculares de la patogénesis viral y determinarán mejoras en el diagnóstico y la terapia.

Palabras clave: Virus Dengue, interferon, IFN α/β, señalización JAK/STAT.

INTRODUCTION

Dengue has reemerged as a global health threat, particularly among children in the tropics of Asia and in young adults as well in the Americas. This mosquito-borne flavivirus causes an estimated 50 million infections annually (1). Most dengue infections result in an illness known as dengue fever (DF). Among DF cases, DENV infections cause dengue hemorrhagic fever (DHF), a vascular leakage syndrome that can lead to dengue shock syndrome (DSS) and death (2) in a less than 0.1%. The complete understanding of dengue pathogenesis of DF and its severe forms, and the progress in developing therapeutic treatment against DENV have been hindered by the lack of biological models that mimic disease progression and by the limited information about gene function related to pathogenesis. In recent years, we have experienced the outcome of biological assays which have been used for the study of antiviral response against DENV and have reveled previously unknown mechanisms of host/virus interactions; but experimental data is often confronted with lack of animal systems or the difficulties inherent in clinical studies.

IFN α/β is made and secreted by mammalian cells rapidly after viral infection, stimulating the onset of an immediate antiviral response in the infected and surrounding cells. The recognition of the IFN α/β network as a fundamental mechanism for the establishment of an antiviral response, and the unveiling of a plethora of mechanisms used by different viruses to counteract this response, have made significant impacts in our understanding of viral pathogenesis (3,4). This elaborate interplay between virus and host is the subject of increasing interest and becoming to be

known as interferon modulation. The existence of numerous sensing mechanisms with different degrees of specificity for viral components ensures that mammalian cells are prepared to recognize viruses that greatly differ in their structural and functional features. The capacity of this array of sensing mechanisms to recognize pathogens is further enhanced by an assortment of interferon-related pathways activated during viral infection, resulting in a complex antiviral response (5,6).

DENV appears to be recognized by most sensing mechanisms of the host cells currently known and activates a strong antiviral response in the host led by IFN α/β production. It has been demonstrated that impairment of IFN α/β signaling in mice (IFNAR$^{-/-}$ IFNGR$^{-/-}$ mice) results in high lethality (7).

Molecular Biology and specifically, gene expression analysis has provided researchers with the identification of genes induced by human host cells in response to DENV (8,9). These gene arrays define a common response observed in many cell types composed of genes involved in the IFN α/β response. Responses of vascular endothelial cells, T cells and dendritic cells are currently investigated in vitro using gene array technology, and activation of key components of the antiviral response can be monitored in patients. Vascular specific responses to DENV include genes involved in the increased endothelial cell proliferation and angiogenesis response, wound healing, cell adhesion changes, T cell inhibition and complement activation. These processes likely participate in the DENV-induced vascular leakage or regulation of the immune response during the acute phase of the disease; and studies discussed in this chapter indicate that key players of the antiviral response of the host against DENV are likely to participate in these pathogenic processes. Among the IFN related response genes, we do no know which genes are particularly specific to DENV, as other RNA viruses could also elicit similar IFN responses. So far, we have attempted to study relevant target cells, like endothelium (HUVECs) as a rational design of studying such responses. We give examples of two genes, ST2 and 2,3 indoleamine dioxygenase or IDO that would be more specific to DENV responses as these two genes were selected between dengue patients and other febrile illness patients (OFI). But, as the complex IFN α/β response associated to DENV infection is unveiled, the mechanisms encoded by the virus to counteract this response have also emerged as a significant area of research. Biological assays have shown that although dengue stimulates a strong IFN α/β response, the virus inhibits IFN α/β signaling (10,11). The understanding of this complex interplay between virus and host may result in a better understanding of dengue pathogenesis and help advance the development of antiviral approaches.

Induction of Antiviral Response. ***FN α/β induction.*** V induces a strong IFN α/β response in natural infections and that IFN α/β is present at high levels for long periods of time in pediatric patients after defervescence (12). The demonstration that impairment of IFN α/β signaling in mice (IFNAR$^{-/-}$ IFNGR$^{-/-}$ mice) results in high lethality and consistent DENV infection in serum and tissue became the demonstration of the essential role of IFN in protecting against DENV infections (7). Global gene expression profiling studies have revealed upregulation of key mediators of the inflammatory cytokine response, the antiviral response through IFNα/β activation, the NF-kB-mediated cytokine/chemokine responses and the ubiquitin proteosome pathway (8,9,13). It has been also shown IFN activation through gene expression analysis of acute phase PMBCs (14).

In addition, dengue induces IFN expression (15) through a pathway involving RIG-I-dependent IRF-3 and PI3K-dependent NF-kB activation, an induction that is likely to occur through TLR7/8/9-mediated recognition of viral RNA (16). Then, dendritic cells, natural targets of DENV infection constitutively expressing TLR7 and IRF7, rapidly lead a systemic IFN α/β response.

The sensing of viral infection can also occur via RIG-I and MDA5 were originally recognized through gene expression analysis (17).

Different players in the JAK/STAT pathway have been found to be antagonized by DENV (10,11); and sequence differences between pathogenic and non-pathogenic strains of DENV possibly correlate with differences in their ability to block IFN α/β signaling (18,19). A schematic representation of the signal transduction events is shown in Figure 1.

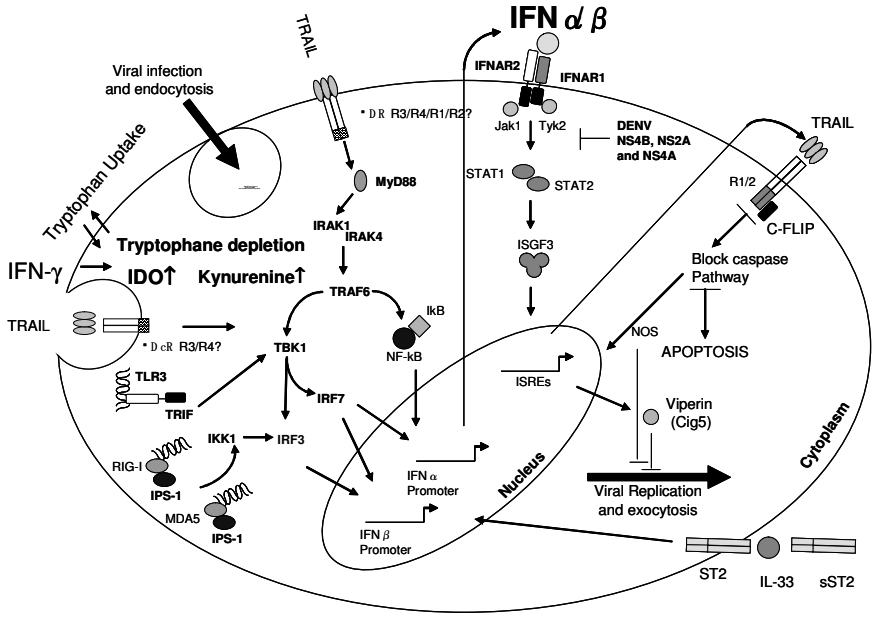

Figure 1. Modulation of IFNα/β in the Host Cell. The recognition of DENV RNA occurs in endosomal compartments during infection, leading to activation of NF-kβ, IFNα/β, IRF7 and IRF3 and activation of IFNα and IFNβ. TRAIL signaling may increase expression of MyD88, resulting in downstream activation of IKKα/β/γ and MAPK cascades and activation of NFκB and AP-1. TLR3 activation through TRIF leads to activation of IFNβ and it is originated from recognition of viral RNA in the cytosol. RIG-I and MDA5 activation results in the stimulation of IRF3, which in turn induces the IFNβ promoter. Secreted IFNα/β activates the IFNα/β receptor and the JAK/STAT pathway, leading to phosphorylation and dimerization of STAT1/2 and formation of the macromolecular factor ISGF3, which translocates to the nucleus and activates ISREs. The nonstructural proteins NS4B, NS2A and NS4A block STAT1 phosphorylation, impair parts of the JAK/STAT pathway and reduces activation of ISREs. Two proteins identified in gene expression analysis, TRIAL and viperin, are represented as part of the IFN response.

DENV mechanisms to block IFN α/β may be somewhat more redundant and complex than so far recognized, with NS4B and other nonstructural proteins blocking interferon in multiple ways, in a manner reminiscent of paramixovirus P gene products (named V, P and C) (20).

By using differential display reverse transcription-PCR (DD-RTPCR), quantitative RT-PCR, and Affymetrix oligonucleotide microarrays in HUVECs infected with DENV, eight differentially expressed cDNAs were identified in 2003: the inhibitor of apoptosis-1, 2'-5' oligoadenylate synthetase (OAS), a 2'-5' OAS-like (OASL) gene, galectin-9, myxovirus protein A (MxA), regulator of G-protein signaling, endothelial and smooth muscle cell-derived neuropilin-like protein, and phospholipid scramblase 1. This analysis revealed an additional 269 gene identities that were upregulated after DENV infection (9). **Figure 2** shows levels of expression (27 genes) found by this Affimetrix microarray in HUVECs, Monocytes, B cells and dendritic cells infected with DENV in vitro.

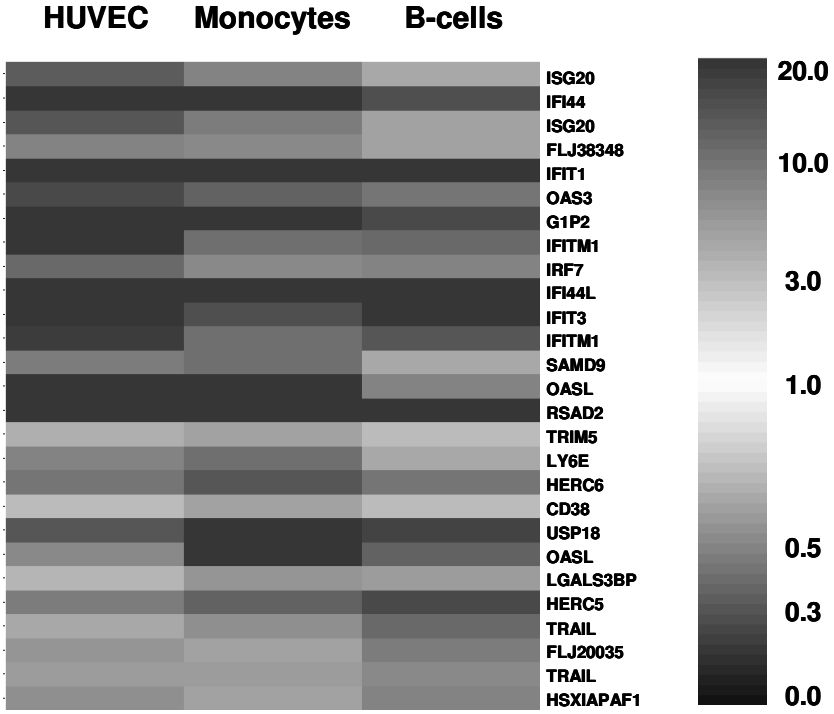

Figure 2. Global gene expression in cells infected with DENV. Expression levels of dengue common response signature (67 genes) in endothelial cells (HUVEC), monocytes (Mo), B-cells (B), and dendritic cells (DC) in vitro. Gene expression levels were normalized to each of the the mock-infected cells (C636 supernatant treatment for 48 h). Normalized expression levels are represented according to the color key shown. Only gene upregulation is shown. Affymetrix GeneChip U133A was utilized and data obtained were analyzed using GeneSpring software (Agilent).

From these genes, 23 are shown in **Table 1** accompanied by their Gene Accession Numbers. Collectively, the aforementioned microarray analysis have provided with a draft of what appears to be the fundamental features of the antiviral response against DENV infection in vitro using primary human cells. The conserved features of the IFN α/β and pro-inflammatory responses observed between the four cell types included in these microarray analysis underscore the importance of innate immune responses during DENV infection.

Table 1. Gene expression signature derived from the common response in endothelial (HUVEC), B Cellos (B), Monocytes (Mo)and dendritic cells (DC) exposed to DENV. The 23 gene IDs, known function and identifications in the Gene Bank are shown. These 23 genes were upregulated in DENV infections in vitro, and confirmed by qRT-PCR.

Name	Functional category	Description	GemBank
G1p2	Anti-viral	Interferon, alpha-inducible protein (clone IFI-15K)	NM 005101
IRF7	Anti-viral	Interferon regulatory factor 7	NM 004030
ISG20	Anti-viral	Interferon stimulated gene 20kDa	NM 002201
OAS3	Anti-viral	2'-5'-oligoadenylate synthetase 3, 100kDa	NM 006187
OASL	Anti-viral	2'-5'-oligoadenylate synthetase-like	NM 003733
RSAD2	Anti-viral	Radical S-adenosyl methionine domain containing 2	A1337069
TRIM5	Anti-viral	Tripartite motif-containing 5	AF220028
HSXIAPAF1	Apoptosis	XIAP associated factor-1	NM 017523
TRAIL	Apoptosis	Tumor necrosis factor (ligand) superfamily, member 10	U57059
CD38	Cyclic ADP-ribose metabolism	CD38 antigen (p45) ecto enzyme	NM 001775
HERC5	HECT E3 Ubiquitin ligase	Hect domain and RLD 5	NM_016323
IFI44	Interferon-Inducible	Interferon-induced protein 44	NM 008417
IFI44L	Interferon-Inducible	Interferon-induced protein 44	NM 006820
IFITM1	Immune suppression	Interferon induced transmembrane protein 1 (9-27)	AA749t01
LGALS3BP	Immune Activation	Lectin, galactoside-binding, soluble, 3 binding protein	NM_005567
USP18	Ubiquitination	Ubiquitin specific protease 18	NM 017414
FW20035	unknown	RNA Helicase/DEAD/DEXD protein Q6PK35	NM 017631
FU38348	unknown	Coiled-coil domain containing 75 protein CCDC75	AV755522
HERC6	unknown	Hect domain and RLD 6	NM 017912
IFIT1	unknown	Interferon-induced protein with tetratricopeptide repeats I	NM 001548
IFIT3	unknown	Interferon-induced protein with tetratricopeptide repeats 3	NM 001549
LY6E	unknown	Lymphocyte antigen 6 complex, locus E	NM_002346
SAMD9	unknown	Sterile alpha motif domain containing 9	NM 017654

As discussed bellow, this Affymetrix analysis was then used as a discovery platform for genes to be tested in patients by qRT-PCR in natural infections with the aim of finding disease markers that are early in the infection phase and also to find pathogenic responses that would correlated with severity of the disease. TNF-related apoptosis-inducing ligand (TRAIL), is up-regulated in HUVEC, dendritic cells and monocytes infected by DENV and its expression is induced by IFN α/β signaling (21) and it was shown as a linker gene between IFN type I and II (IFN α/β IFN λ)

Due to the lack of animal models, the microarray approach to study DENV illness met it's more direct application in providing gene expression profiles in blood samples from patients with DF and DHF. These studies have revealed an antiviral response, and identified potential markers of disease (8,14,22). The chemokines IP-10 and I-TAC (both ligands of the CXCR3 receptor) have also been reveled in microarray studies of DENV-infected human cells as one of the prominent responses found in patients (8) along with CCL8 or MCP-1.

Since these proteins could be detected early after the onset of fever in dengue patients, this finding may have implications in human disease and early prognosis (8,22). Viperin (Cig5) is highly unregulated during DENV infections in Rhresus and Humans (8,9,13) and it's role as an IFN-induced gene that reduces viral release has been described for other viruses (23,24). In a recent global gene expression arrays analysis(Amersham/GE) in PBMCs from 30 patients with DF or DHF, 47% of all altered genes were part of the interferon response; and the majority of the interferon-induced genes were strongly up-regulated in DF compared with the DHF patients, suggesting a significant role of the interferon system during DENV infection and possibly indicating (14).

A robust production of IFN α/β in PBMCs collected from DF patients was confirmed by ELISA (14). A similar tendency was also found in DF cases compared to DHF cases in an alternative study in Vietnam utilizing Illumina arrays (Cameron Simmons, personal communication). When mRNA levels of paired samples from acutely infected DF and DHF patients are compared using qRT-PCR, a dynamic range in the expression levels of the following genes was observed: CCL8 (MCP-2), CCL2, IP-10 (CXCL10), IDO, IRF-7, LAMP-3, TRAIL (TNFSF10), USP18, PLSCR1, G1P3. Remarkably, a large increase in expression of genes involved in immune response was detected in DF vs. DHF by independent laboratories (14; Cameron Simmons, personal communication). Moreover, Vietnamese children with DSS had substantially lower transcriptional activity of multiple IFN-stimulated genes (ISGs) than did children presenting with DHF without shock (22).

We have observed too that an overall decrease in the Interferon stimulated genes are down-regulated in DHF compared to DF case over the time of the acute phase of disease usin qRT-PCR arrays (data not shown). Another common finding in gene expression profiles in whole blood or PBMCs, is the elevated expression of IFIT2 in DSS patients and CCL5 and TNFSF13B in DF patients (14,22). In the study of Ubol et al. (14), interferon inducible genes that were up-regulated in patients with DF included GBP1, IFI27, IFI44, IFIH1/mda5, IFIT1, ISG15, Mx1, and CXCL10/IP10. In contrast to that finding, GBP1 and Mx1 were associated in an early screen utilizing cDNA based-arrays for DSS cases (22). These findings could indicate the

exhaustion of immune cells in the more severe condition of the disease or the result of interferon antagonistic functions displayed by viral components during infection (discussed further below).

To further evaluate the specific response during dengue infections, a comparative study between DF patients and patients with other febrile illnesses was performed, and the results indicated that ST2 and IDO were significantly increased in patients with DF with respect to patients with other febrile illnesses and have further confirmed that ST2 is present in acute dengue infections (**Table 2**). In addition, we included genes that were very specific to HUVEC response to DENV, not observed with other flaviviruses tested in parallel to DENV. Among these genes were ST2 and IDO were detected as highly regulated genes.

Dynamic changes in gene expression can occur over the course of illness, which can be observed by qRT-PCR profiles and by Illumina chip hybridization. Therefore, comparisons of gene expression profiles between samples and platforms need to be carefully done. Due to the lack of post-febrile illness gene expression data, our understanding of the antiviral

response is solely based on comparisons during acute illness, and we hope to overcome this limitation by conducting prospective clinical studies.

Table 2

Genes	CD8 Acute	B Acute	Mo Acute	CD4 Acute	DF (PBMC)	DHFI (PBMC)	DHF2 (PBMC)
IF127	52.79	32221.5	31494	22049.4	0.67	1.27	0.78
IF144	3.24	13.97	90	24.66	0.11	0.15	0.27
IFIT1	10.36	92.99	173	22.44	0.12	0.14	0.26
IL1RL1/ST2	5.85	5.09	1.48	5.04	**6.64**	**4.98**	**3.84**
ISG20	0.94	2.31	82.8	9.43	0.32	0.58	0.37
IDO	637.3	9849.4	12650		**303**	**932**	**482**
IRF7	2.41	9.18	8.4	18890.5	0.32	0.43	0.3

Table 2. Quantitative RT-PCR array shows the levels of expression of RNA derived from CD8, B, Monocytes and CD4 cells derived from patients with acute febrile illness. StemCell Technology negative selection kits were utilized to obtain each of these cell types from anti-coagulated blood at febrile state. Then, PMBC RNA expression profiles from patients at DF and DHF state of disease was normalized to RNA expression levels from PBMCs obtained from patients with a febrile illness and confirmed negative for DENV infection. From that normalization, two genes, ST2 and IDO show specific upregulation and therefore are shown as more specific to the dengue virus infection. Other genes shown here were prominently expressed in dengue acute infections normalized to normal cells from un-infected individuals.

As we move forward in this field with larger sample numbers, collected early and across a breadth of clinical disease states, we will learn more about the dynamic interaction between the host and the virus. Future studies should

attempt multi-parameter analysis of host gene expression data by bringing in information on the infecting virus, via viral genomic sequence analysis or biological characterization. Furthermore, gene expression studies have to date been descriptive in nature. Attempts to identify and prospectively test possible prognostic markers for severe disease should be encouraged.

The results of these studies, despite the differences in the systems used to assess levels of gene expression, remarkably converge in several common genes. This is very encouraging, given the variability observed in other clinical data sets such cancer microarray comparisons. Very strong similarities were detected mainly in the broadly prominent "immune response" of patients in the febrile stage of a dengue illness. Chief amongst these are: a) transcripts from IFN α/β inducible genes and signaling molecules of the IFN α/β pathways, b) transcripts from select chemokines (IP-10, MCP-1 and MCP-2 in particular), c) protein ubiquination and d) cell survival and apoptotic signaling pathways. The possible roles of these gene products in controlling the spread of the disease and the inflammatory response are currently under analysis. In addition, possible correlates between these up-regulated gene products and disease severity are being investigated. In particular, we would like to summarize the genes for which mRNA levels have been confirmed by qRT-PCR after their discovery in Amersham/GE, Affymetrix or Illumina chip platforms.

Tumor necrosis factor related apoptosis inducing ligand (TRAIL) From the dengue common response signature, characterized by the IFN α/β signaling network, one gene, TRAIL, has been identified as a central player in the antiviral response and can be proposed as a potential common linker of the IFN α/β inducible genes. A protective physiological role for TRAIL as an antibacterial agent has been indicated (25). TRAIL is a member of the TNF family that is specifically involved in neuroprotection and growth proliferation in non-cancer cells (25-28). TRAIL appears to promote apoptosis in cancer cells by activating the death receptors DR4 and DR5 (29) and negatively regulates innate immune response independent of apoptosis (30). *In vitro* and *in vivo* studies have demonstrated tumoricidal and anti-viral activity of TRAIL without significant toxicity towards normal cells or tissues (31).

Interferons enhance expression of TRAIL, while on the other hand, TRAIL treatment can enhance expression of IFN-inducible genes like Oligoadenylate synthase or OAS, RNA helicase MDA-5, IFITM1, IFIT1, STAT1, LGal3BP, PRKR, TRAIL gene itself, and as described by others, in tumor cells, IFN-ß itself was induced (32). The down-regulation of MCP-2 and IP-10 by TRAIL has been reported in HUVECs, causing reduced pro-inflamatory response (33). The molecular cross-talk and functional synergy observed between TRAIL and interferon signaling pathways may have implications for the physiologic role and mechanism of action of TRAIL protein during infection.

Recombinant TRAIL (rTRAIL) treatment strongly inhibited DENV replication and DENV antigen levels in dengue-infected dendritic cells by an apoptosis-independent mechanism (21) (Warke et al., 2008). Furthermore, rTRAIL treatment of DENV-infected dendritic cells inhibited the expression of pro-inflammatory cytokines and chemokines (IL-6, TNF α, MCP-2, IP-10, MIP-1ß) (unpublished data). These data suggest that TRAIL plays a beneficial role of anti-viral and pro-inflammatory cytokine suppression during DENV infection.

Further investigation of TRAIL as an anti-inflammatory protein in the context of DENV infection will help better define its role in controlling the pro-inflammatory response triggered after DENV infection.

Interleukin-1 receptor-like 1 precursor (IL1RL1)-ST2 gene. IL-1RL1 / ST2 is a member of the interleukin-1 receptor (IL-1R) family of proteins. Alternative splicing of the gene generates three mRNAs, corresponding to a longer membrane-anchored form (ST2L), a shorter released form (sST2) and a membrane bound variant form (ST2V) (34-36). ST2L has been found to be selectively expressed on Th2-polarized T lymphocytes and mast cells and has been described as an activation marker for Th2 cells (37,38). It has been shown that sST2 can inhibit IL-1R and TLR4 signaling, through the sequestration of MyD88 and Mal proteins (39).

Pro-inflammatory stimuli, including LPS and cytokines, induce the expression of sST2 in human and mouse in vitro models (40-42). The administration of sST2 or ST2-Fc fusion protein is able to suppress the production of pro-inflammatory cytokines in vitro and in vivo and recently reviewed (39,43) attenuate the inflammatory response in vivo elevated levels of sST2 have been found in diseases including Th2-associated inflammatory disorders, autoimmune diseases, asthma, sepsis, and myocardial infarction (44-50); and more recently it's significance in DENV infection has been revealed (51).

The ST2L molecule was recently described as part of receptor complex for the cytokine IL-33 (52,53). A dual role has been suggested for IL-33: as a nuclear factor with transcriptional regulation activity and as a pro-inflammatory cytokine (54). In a mouse model of cardiac disease the beneficial anti-hypertrophic effect of IL-33 is blocked by sST2, suggesting that this protein could be acting as a decoy receptor (48). ST2 and IL-33 binding recruits MYD88, IRAK1, IRAK4, and TRAF6, followed by phosphorylation of MAPK kinases (54). In a small cohort of patients, mostly classified as DF, sST2 levels in serum from DENV infected

patients were found to be higher than in patients with other febrile illnesses we found; we also found that the levels of sST2 were higher in secondary infections compared to primary infections (51).

The increased levels of sST2 were observed at the late febrile stage and especially at defervescence. We also found correlations in dengue patients between sST2 levels and other parameters associated with disease severity. These results prompted us to propose that serum levels of the sST2 protein could be a marker for dengue infection, a parameter that could indicate dengue infection when the levels of circulating virus are dropping. Serum sST2 levels could also be an indicator of the inflammatory response and, as suggested by others (55) could be a down-regulatory mechanism triggered to control the exacerbated inflammatory response.

Specific endothelial response to DENV: Indoleamine 2,3-dioxygenase (IDO) IDO was detected as a strongly induced gene after dengue infection of several primany human cells (9). When IDO expression levels were determined in PMBCs of patients infected with DENV, and compared to the levels of expression of PMBCs from patents with other febrile illnesses PMBCs, IDO was one of the genes that appear to be more specific to dengue infected patients. Therefore we decided to study this enzyme in more detailed in vivo. IDO is an enzyme ubiquitously distributed in mammalian

tissues and cells including dendritic cells (56) and T cells (57). It catalyzes the initial and rate-limiting step in the catabolism of L-tryptophan along the kynurenine pathway (58).

In vivo, IDO activity in serum is increased under pathological conditions such as toxoplasmosis (59), viral, and bacterial infections (60,61), allograft rejection (56). IFNγ is the most potent known inducer of IDO expression (62). IDO-expressing cells can inhibit T cell proliferation and function by depleting L-tryptophan in the surrounding microenvironment. This process is termed "immunosuppression by starvation" (63). Further studies will be needed to investigate whether tryptophan metabolites released by DENV infected endothelial cells are involved in inhibiting CD8 T cells.

We hypothesize that IDO is involved in the establishment of an immunosuppressive condition during DENV infection, as described by others (55).

Gene expression analysis showed that kynureninase up-regulation in endothelial cells infected with DENV was elevated. This finding suggested that IDO (the rate limiting step in the catabolism of Tryptophan) might play a role in DENV induced pathophysiology. Using Mass Spectrometry methods, reduced levels of L-tryptophan and increased levels of kynurenine were found in DENV-infected patients compared to other febrile illnesses, during the acute stage of the disease. This result supports the hypothesis of T and other immune cell inhibition during dengue infection.

Conclusions and Perspectives Molecular Biology has served to study the gene expression profiling studies which have recently revealed key players of the cell response to DENV infection. The data from different research groups converge in three mayor categories of gene expression patterns. The NF-κB-mediated immune response, the IFN α/β network and the ubiquitin proteosome pathway. The up-regulation of IFN α/β is accompanied by high expression of pro-inflammatory cytokines and complement inhibitors. Studies have recently focused on the differences between DF and DHF. Interestingly, during acute-phase infection, the IFN α/β response represents a substantial subset of differentially regulated genes in PBMCs from acutely ill patients, and this IFN α/β response seems to offer more protection for infection in patients with DF than in patients with DHF. Further studies should be conducted in a time dependent manner during disease progression and, if possible, using isolated subsets of blood cells.

The significant upregulation of IFN α/β inducible gene products found in patients with DF is consistent with the role of IFN α/β in controlling viral replication and the lower viral loads reported in patients with DF compared to patients with DHF. In analyzing gene expression profiles, we have unrevealed key players of IFN α/β modulation which could play a role in disease pathogenesis, including endothelial damage, inflammatory response and capillary leakage. Their differential regulation during the course of illness, as mentioned above needs to be further analyzed. The subversion of the antiviral response by DENV needs to be further explored in the context of disease progression and in light of the recently identified, key players of IFN α/β modulation.

The increased knowledge that we have gained in recent years have also resulted in the identification of potential markers of disease severity; some

of which are induced as part of the IFN α/β response. Their potential in defining risks for severe illness needs to also be further assessed over the course of acute disease in patients. The lower IFN α/β response in DHF patients could be a reflection of the antagonistic function of DENV NS4B and other non-structural proteins. The specific molecular interactions between DENV protein products and the IFN α/β pathway need to be further studied. In addition, the subversion of IFN α/β response by DENV needs to be understood in the context of illness.

Studies will focus on concrete molecules recently identified in gene expression profiles and how they are modulated over the course of disease. A more precise definition of these molecular interactions will bring along studies towards therapeutic discovery. More research will likely be centered in identifying DENV strains with differences in their ability to block IFN α/β signaling as a systematic approach to better understand virulence and develop attenuated vaccines for efficacy and safety studies.

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