University of Nevada, Reno

Dengue Fever: An Examination and Case Study

A thesis submitted in partial fulfillment
of the requirements for the degree of

Bachelor of Science in Chemistry and the Honors Program
by

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Dengue Fever: An Examination and Case Study

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Abstract

Dengue Fever is a mosquito-borne disease found primarily in tropical regions of the Earth. Although not prevalent in much of the western world, dengue appears to be an emerging virus, which prompts a need for further understanding. The purpose of this study is to provide a general overview of the virus, followed by a closer examination of one of its molecular modes of action. A constructed case study is also provided, in order to characterize how the virus could potentially manifest and be treated in the United States.

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Chapter 1: An Overview of Dengue

Abstract

Dengue (den-gee) Fever (DF) is a mosquito-borne disease caused by the dengue virus. The virus belongs to the family *Flaviviridae* and genus flavivirus.[1] Like other *flaviviruses*, such as yellow fever virus and West Nile virus, the dengue virus utilizes single stranded RNA as its genetic content.[1] The disease is found primarily in tropical and –subtropical regions that support the life cycle of the mosquito.[2] Once infected, signs and symptoms of the disease vary from mild febrility to shock and death; however, prevention of infection and early treatment can significantly reduce both morbidity and mortality.[3] Although no vaccine or drug treatments currently exist, some viral proteins thought to increase pathogenicity of the virus have been identified.[1] These proteins could potentially act as the target of drug and vaccine development.

Introduction

The dengue virus is a *flavivirus* that utilizes positive, single stranded RNA and a mosquito vector.[1] Female mosquitoes of the genus Aedes (specifically, *Aedes aegypti and Aedes albopictus*) are known to transmit the virus.[1] The mosquito must first acquire the virus by feeding on an infected host while a significant amount of virus is present within the blood.[2] For the host, this typically occurs prior to symptom onset.[2] After the blood meal, the virus requires approximately 8-12 days to incubate before it can be transmitted to a new host.[2] The mosquito can transmit the virus more than once, since it will remain infected for the remainder of its life.[2] In some instances, human to human transmissions have occurred through direct contact with blood, as occurs during organ transplantation, blood transfusion, and placental blood exchange.[2]

After becoming infected, the host will remain symptomless for about 4 to 6 days.[4] During this period, the virus is incubating and growing in number. During this time frame, an uninfected mosquito can pick up the virus during a blood meal and then transmit it to a new host.[1] After the incubation period, the period of illness commences and runs its course for about 3 to 10 days.[2] Signs and symptoms are generally flu-like.[3] Patients typically report severe headaches, muscle pain, joint pain, pain behind the eyes, nausea and vomiting.[1] Signs include an elevated body temperature characteristic of high fever (40 degrees Celsius or 104 degrees Fahrenheit), skin rashes, and swollen glands.[4] Typically, signs and symptoms are mild; however, individuals with weakened immune systems prone to secondary infection or subsequent/multiple infections (by the multiple dengue serotypes) are at high risk of developing dengue hemorrhagic fever (DHF), a severe form of the disease.[3] This complication is characterized by a drop in temperature (below 38 degrees Celsius or 100 degrees Fahrenheit), as well as damage to circulatory and lymph tissue, bleeding (typically from the nose and gums), plasma leakage, edema, liver enlargement, organ impairment.[4] Rapid breathing and blood in vomit, urine, and stool may be observed.[4] The patient may report debilitating abdominal pain, fatigue, and frequent vomiting.[4] In concert, these issues can lead to dengue shock syndrome (DSS), which is characterized by high blood loss followed by shock and death.[3] Additionally, the dengue virus or anti-dengue virus antibodies can be detected through a blood test.[3]

Currently, there are neither vaccines that build immunological memory nor drugs available to treat DF.[1] After successfully recovering from the disease, naturally acquired active immunity will be built, but only to the serotype that was present during infection and illness; immunological memory to one serotype does not translate to another.[1] As blood and fluid leakage is of primary concern, maintenance of proper body fluid volume is critical.[3] Pain

relievers containing acetaminophen can be used; however, aspirin and other anti-inflammatory medications should be avoided, since they can increase hemorrhaging.[3] Perhaps the best form of treatment is prevention, which involves taking measures to reduce contact with mosquitoes and controlling mosquito populations. Repellants should be used both outdoors and indoors; garments should cover as much skin as possible; doors and windows should have impassable screens; waste should be disposed of properly; insecticides should be applied appropriately.[4] Additionally, objects that could serve as mosquito breeding sites (containers that can hold stagnant water) should be frequently maintained, periodically drained of water, or removed.[4] Precautions should also be taken to avoid mosquito bites while in the presence of another person infected with the virus.[3] Early detection and treatment can also significantly reduce the risk of further complications and death.[3]

As the dengue virus employs a mosquito vector, the prevalence and incidence of the disease increases in environments that prove conducive for the life cycle of the insect. The virus will be particularly prominent in urbanized areas during periods of optimal rainfall, when more stagnant water is made available for the mosquitos to lay their eggs in.[4] Subsequently, the dengue virus is largely endemic to tropical areas, such as Southeast Asia, The Pacific Islands, The Caribbean, Mexico, Africa, and Central and South America.[3] However, there have been occasional cases reported within the United States.[2] Many of these incidents occur after an individual has travelled to a tropical area, become infected, and then returned to the U.S.[2] Historical data supports dengue's status as an emerging disease.[2] In the 1950s, DHF was documented in the Philippines and Thailand during epidemics.[2] After 1981, DHF was observed in regions of South and Central America.[2] This spread has been attributed to the large amount of global traffic since the outbreak of World War II.[2]

Dengue epidemics can occur when large numbers of people without immunity to one or more of the four dengue serotypes (DENV 1, DENV 2, DENV 3, and DENV 4) coincide with large numbers of mosquitoes, making contact and transmission more probable.[2] Current estimates suggest that dengue is endemic to at least 100 countries, with 3900 million people at risk of infection. Around 50 to 100 million dengue virus infections are reported per year, with 22,000 deaths.[4]

Body of Review

Absence of effective drug and vaccine treatments is a concern that should be addressed. Evidence suggests that the second serotype of the dengue virus (DEN-2) produces certain proteins that contribute to its pathogenicity. These proteins likely function by inhibiting certain aspects of the interferon-mediated, anti-viral response. Ascertaining the function and mechanisms of these proteins would prove useful for not only understanding the dengue virus, but also developing effective treatment against it. As dengue is an emerging disease, the need for such treatments has become more crucial.

Conclusion

Although dengue fever itself has low mortality and morbidity, its severe forms – dengue shock syndrome and dengue hemorrhagic fever- are more life-threatening. Subsequently, available treatment methods may not entirely suffice, since they address the effects of the disease (ex: low fluid volume) rather than its cause. In addition, *Aedes* mosquitoes are fairly adaptable to environmental challenges and human efforts, so managing mosquito populations may not be an entirely reliable method to preventing infection.[1] Specific attention should be given to the virus's polypeptides. Such focus may shed light on the mechanisms by which the polypeptides suppress

or evade the host immune response, thereby augmenting the pathogenicity of the virus. By targeting these proteins, the cause of the problem can be addressed. The following research identifies three key proteins that contribute to the virus's pathogenicty, and aims to understand their effects on the immune system.

Chapter 2: Inhibition of Interferon Signaling and Response by Dengue Virus Proteins

Abstract

Dengue virus possesses a positive strand RNA genome.[5] Translation of RNA and subsequent cleavage by peptidases results in 10 protein products (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).[5] Preliminary evidence suggests that some of these proteins aid the virus by hindering the IFN mediated response, thereby preventing host cells from triggering an anti-viral state.[5] The experiments performed support this assertion. NS2A, NS4A, and NS4B were found to augment viral replication.[5] NS4B, NS2A, and NS4A were found to down-regulate IFN stimulated gene expression of anti-viral protein products.[5] Finally, it was shown that NS4B blocked IFN signaling via the Jak-STAT pathway.[5] As dengue is an emerging virus, elucidating the mechanisms by which these viral proteins act may prove useful in producing antiviral compounds to combat the pathogenicity of the virus.[5]

Introduction

Translation of dengue's positive strand RNA results in the formation of a 3,391-aa-long polypeptide.[5] Cleavage by both host and virus peptidases forms ten protein products- 3 structural (C, prM, and E) and 7 non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5).[5] NS3 and NS5 were shown to be involved in viral RNA replication; the remaining 5 non-structural proteins appear to inhibit the interferon response in some way.[5]

The immune response begins when the virus enters the host cell and commences replication of its genome and synthesis of its proteins.[5] Transcription factors, such as IFN regulatory factor (IRF)-3, IRF-7, NF-kB, and activating transcription factor 2 (ATF2)/ c-Jun are activated by viral components.[5] These transcription factors stimulate the expression of IFN

alpha/beta, which bind to the IFN alpha receptor (IFNAR) displayed on infected cells.[5] The binding event triggers the Jak-STAT signal transduction pathway, which eventually results in the transcription of genes coding for IFN-stimulated regulatory elements (ISRE).[5] Activation of around 100 of these genes induces an anti-viral state.[5]

Dengue viral proteins presumably disrupt one or more aspects of this immune response.[5] Other viruses, such as the hepatitis C virus (HCV), also display anti-IFN mechanisms, further suggesting the dengue virus's own IFN inhibition.[5] However, HCV anti-IFN mechanisms cannot necessarily be compared to the dengue virus's response, as the two don't possess adequate sequence similarity.[5] Further tests must be conducted on the non-structural viral proteins to deduce underlying mechanisms.[5]

Methods

Constructing Plasmids, Acquiring Virus Strains, and Establishing Cell Lines

DEN-2 infectious cDNA clones (pD2/IC-30P-A) were utilized to clone the 10 viral proteins.[5] Mammalian pCAGGS was used as a vector to clone genes. Hemaglutitin (HA) tags were placed at 3' end of genes to ensure production of DEN-2 proteins with HA tag at C-terminal.[5] Reporter plasmids (pHISG-54-CAT and pISRE$_4$-9-27-CAT with ISRE promoters were utilized.[5] pkB-luc, pRL-TK-luc, and peak-8 plasmid were used. GFP-labeled Newcastle disease virus (NDV-GFP) was constructed and grown.[5] 10-day-old chicken embryos were inoculated with Sendai Virus.[5] A549, LLCMK2, Vero, and 203T cells were obtained and kept in DMEM containing 10% FBS. Chicken Embryo Fibroblast (CEF) were obtained and kept in MEM with 10% FBS.[5]

"Transfection and NDV-GFP Infection of CEF and A549 cells"[5]

CEF and A549 cells were transfected, incubated, and infected with NDV-GFP. The cells were again incubated, before GFP expression was visualized by fluorescence microscopy.[5]

"Reporter Gene Assays"[5]

293T cells and Vero cells were transfected with either pHISG-54-CAT or pISRE$_4$-9-27-CAT, pCAGGS-FL, and pCAGGS.[5] After twenty-four hours, cells were either treated or mock treated; 292T cells were infected with Sev and Vero cells were incubated with human IFN-B.[5] Twenty-four hours after this treatment, the cells were collected and lysed. CAT and luciferase assays were performed.[5]

"**Immunofluorescence**"[5]

Cells were grown and fixed on microscope coverslips.[5] Dengue mouse antibody, HA-TAG mouse antibodies, phosphorylated STAT1 rabbit polyclonal antibody, and rabbit polyclonal anti-calnexin were employed as primary antibodies.[5] The secondary antibodies were Texas-red and FTTC-conjugated anti-rabbit or anti-mouse antibodies.[5] Nuclear chromatin staining was conducted.[5]

Results

The genes encoding the 10 DV polypeptides were amplified with PCR and cloned into the pCAGGS(3'HA) vector to produce plasmids with DEN-2 proteins tagged at the C-Terminus with a HA tag.[5] Since IFN is produced after transfection of plasmid DNA, the replication of IFN sensitive viruses (such as NDV) is inhibited.[5] IFN antagonists should enhance replication of these IFN sensitive viruses.[5] Subsequently, CEF was transfected with the 10 DEN-2 plasmids and then infected with NDV expressing GFP.[5] Fluorescence microscopy was utilized to visualize

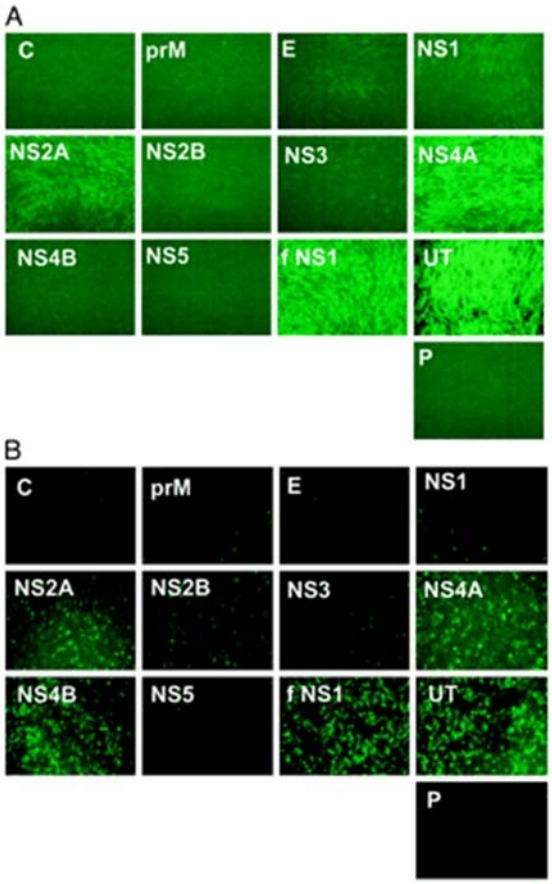

Figure 1: A) When visualized with fluorescence microscopy, CEF transfected with NS2A and NS4A showed greater degrees of fluorescence. CEF transfected with fNS1(influenza A virus NS1 protein) and untransfected cells also showed significant amounts of fluorescence. B) A549 cells showed similar patterns of fluorescence, with the addition of NS4B transfected cells also showing noteworthy levels of fluorescence.

Acknowledgement: Munoz-Jordan et al.

fluorescence and indicate the degree of NDV replication in the presence of various DEN-2 proteins.[5] Based on these results, it could be ascertained which DEN-2 proteins are antagonists.[5]

This screening method was also adapted to human A549 cells.[5] In CEF, NDV-GFP replication was enhanced in the presence of DEN-2 NS2A and NS4A expression plasmids (Figure 1A).[5] In human A549 cells, NDV-GFP replication was enhanced in the presence of NS2A, NS4A, and NS4B (Figure 1B). [5]

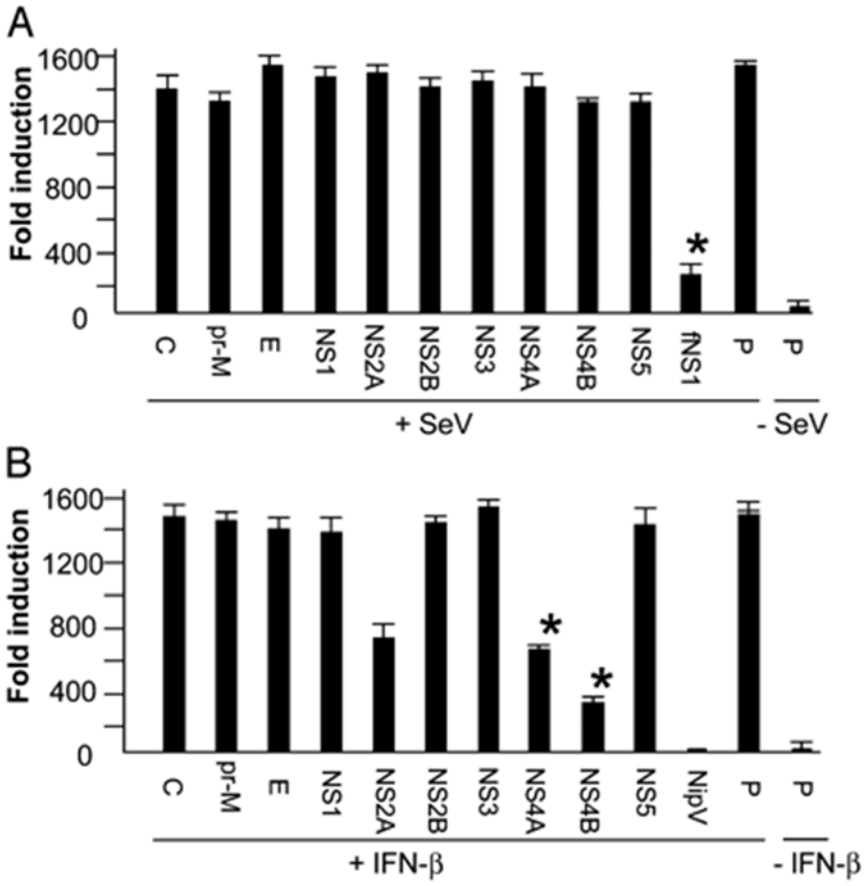

Figure 2: A) Fold induction of cells transfected with DEN-2 protein expression plasmids is very similar to fold induction of cells transfected with empty plasmid (control). This indicates minimal/no inhibition of IFN induction. Cells transfected with the fNS1 protein expression plasmid (fNS1 is another control) showed significant decreases in fold induction, which indicates inhibition of IFN induction by Influenza A. B) Fold induction of cells transfected with NS4B, NS2A, and NS4A protein expression plasmids were markedly lower than fold induction of cells transfected with empty plasmid. This indicates that these proteins inhibit IFN alpha/beta-mediated signal transduction and stimulation of the ISRE-54 promoter.

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The previous experiment indicated that particular DEN-2 proteins enhance viral replication, but didn't indicate if and how they inhibited the IFN response.[5] To determine this, the ability of the DEN-2 proteins to block IFN induction and/or IFN-stimulated signal transduction was analyzed.[5] 293T cells were transfected with an ISRE-CAT plasmid and pCAGGS plasmids expressing DEN-2 proteins.[5] Empty pCAGGS plasmid was used as a control to indicate basal expression of the ISRE reporter gene.[5] Twenty-four hours after infection with SeV, CAT activity was detected.[5] CAT activity indicated that the ISRE-54 promoter was activated, which suggests that the DEN-2 proteins did not inhibit IFN induction in 293T cells (Figure 2A).[5]

The ISRE-54 promoter can be stimulated by the IRF-3 transcription factor and by the IRF-9 transcription factor.[5] In the latter scenario, IFN alpha/beta stimulates the promoter through activation of STAT1/STAT2/IRF-9 (IFN mediated signal transduction).[5] To assess the capacity of the DEN-2 proteins to inhibit IFN-mediated signal transduction, ISRE-54 promoter activation was measured in Vero cells transfected with DEN-2 protein expression plasmids (and ISRE-54-CAT reporter plasmid).[5] The transfected cells were incubated with IFN beta for 24 hours, lysed, and assayed for CAT activity.[5] NS4B, NS2A, and NS4A reduced Cat expression, which suggests these proteins interfere with IFN-mediated signal transduction in Vero cells (Figure 2B).[5]

The individual and combined effects of the three DEN proteins were tested.[5] Vero cells were transfected with HA-tagged NS2A, NS4A, and NS4B plasmids, as well as a plasmid containing the ISRE-9-27 promoter.[5] Additionally, some cells were transfected with all three DEN protein expression plasmids.[5] Cells were treated with IFN and then assayed for CAT

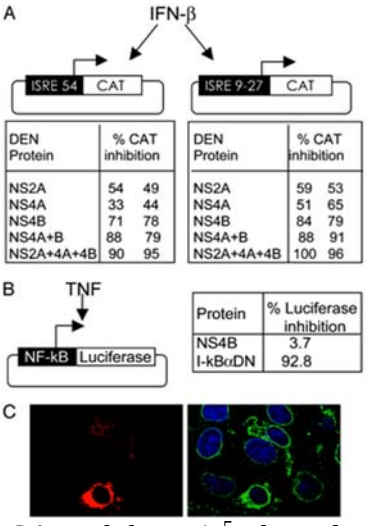

Figure 3: A) Each DEN protein resulted in significant CAT inhibition, with NS4B resulting in the most. The combined effects of all three were stronger than what was achieved by any individually. B) Luciferase inhibition is minimal when NS4B is expressed, which indicates NS4B does not inhibit TNF signaling significantly. C) NS4B fluoresces within vicinity of calnexin, an ER protein. This indicates that NS4B associates near the ER.

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activity (measured in % of CAT inhibition).[5] The individual proteins yielded values similar to those obtained when the ISRE-54 promoter was used (Figure 3A).[5] It was also observed that the combined effects of the three DEN proteins were greater than the isolated effects (Figure 3A).[5] Immunofluoresence was used to visualize the intracellular location of the cells. It was found that the proteins associate with the ER (Figure 3C).[5]

Focus was shifted to NS4B and its specificity for IFN signaling, since it displayed the strongest inhibition of the three DEN proteins.[5] NS4B's ability to inhibit activation of NF-kB by TNF was tested.[5] Vero cells were transfected with plasmid containing a promoter responsive to NF-kB.[5] The promoter drives the expression of firefly luciferase (FL).[5] The cells were co-transfected with pCAGGS-NS4B-HA.[5] A plasmid expressing IkBaDN served as a positive control for the repression of NF-kB.[5] The cells were then incubated with TNF.[5] FL and Renilla Luciferase (RL) activities were determined.[5] Results showed that FL was not affected by

NS4B.[5] This indicates that NS4B does not inhibit the TNF signaling pathway and that it does not cause a general inhibition of other signaling pathways.[5] NS4B seems to be specific to IFN signaling.[5] Also, immunofluorescence indicated that NS4B, NS2A, and NS4A localize near the ER.[5]

To ascertain how IFN signaling is inhibited, focus was shifted to NS4B's effect on the activation of STAT1.[5] IFN-alpha/beta bind to the IFN-alpha receptor, activating JAK1 and TYK2 kinases.[5] These phosphorylate STAT1 and STAT2, which form a heterodimer and move to the nucleus.[5] Another mechanism of STAT phosphorylation and nuclear translocation involves IFN-gamma.[5] When IFN-gamma binds to the IFN-gamma receptor, the JAK1 and JAK2 kinases become phosphorylated.[5] These kinases then phosphorylate STAT1, which forms a homodimer.[5] The homodimer moves to the nucleus and stimulates transcription of genes.[5]

LLCMK2 cells were transfected with either empty plasmid or plasmid expressing HA-tagged NS4B.[5] The cells were then treated with IFN beta, before being visualized with immunofluorescence.[5] Nuclear staining was not seen in cells not treated with IFN beta and in the majority of IFN treated cells expressing NS4B.[5] The lack of staining indicates a lack of phosphorylation.[5] In contrast, cells with the empty plasmid showed staining, indicating phosphorylation.[5] This shows inhibition of IFN-alpha/beta mediated signaling by NS4B (Figure 4A).[5] A similar experiment was carried out, where cells were treated with IFN-gamma.[5] It was found that NS4B also block IFN-gamma mediated signal transduction (Figure 4A).[5]

To determine whether NS4B contributes to IFN signaling inhibition during a DEN-2 infection, LLCMK2 cells were infected with DEN-2 for six days, before given either IFN-beta or IFN-gamma treatment.[5] The cells were then visualized using immunofluorescence[5]. It was found

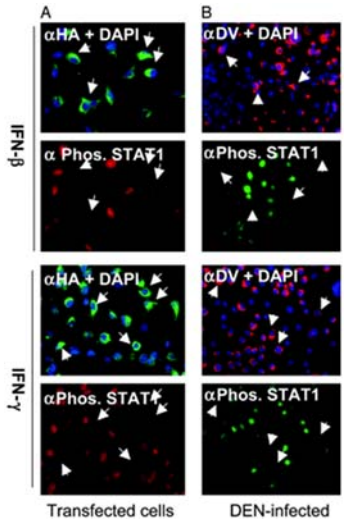

Figure 4: A) Cells expressing NS4B (green) displayed no phosphorylation (no red) following both IFN-beta and IFN-gamma treatment. Cell not expressing NS4B displayed phosphorylation (red). B) Cells expressing DEN-2 proteins (red) showed no phosphorylation (no green), while cells not expressing DEN-2 proteins showed phosphorylation (green).

that most infected cells did not show phosphorylated STAT1 after both treatments, while all uninfected cells showed phosphorylation of STAT1.[5] This suggests that NS4B's inhibition of IFN signaling contribute to the pathogenicity of DEN-2 (Figure 4B).[5]

Discussion

The results present two lines of evidence which support the notion that DEN-2 proteins NS2A, NS4A, and NS4B are IFN antagonists.[5] The first line of evidence involved the NDV-GFP assays.[5] The NDV-GFP assay was used to detect potential IFN antagonists.[5] Of the ten DEN-2 polypeptides, three (NS2A, NS4A, and NS4B) were shown to augment NDV-GFP replication in

human A549 cells, while only two (NS2A and NS4A) accomplished a similar effect in CEF.[5] Since NS2A, NS4A, and NS4B facilitated viral replication, it is likely they are IFN antagonists.[5] The results of the assay also suggest species specificity in the actions of DEN-2 proteins.[5] The specific mechanisms which lead to differences in the IFN response and DEN-2 protein effects on the IFN response have not been fully elucidated and should be the subject of further research.[5]

The second line of evidence stems from the reporter gene assays.[5] It was shown that NS4B, and to a lesser degree NS2A and NS4A, inhibited the activation of two distinct ISRE promoters in response to IFN-beta.[5] In concert, these proteins inhibited activation to a far greater degree.[5] Immunofluorescence indicated that these proteins associate closely to the ER, which further suggests the possibility of some interactions.[5] The nature of these interactions is not clearly understood, and should be explored in future research.[5]

It was also shown that in the presence of NS4B, STAT1 showed little phosphorylation.[5] This indicates that NS4B intereferes with IFN signaling; however, the specific mechanism of action is unknown.[5] It is possible NS4B takes effect on Jak kinases, preventing phosphorylation all together.[5] Alternatively, NS4B could degrade already phosphorylated STAT1.[5] NS4B's interactions with key proteins in the IFN signaling pathway should be further explored.[5]

The results indicated that the DEN-2 proteins, particularly NS4B, inhibit the IFN response by interfering with signaling.[5] However, this is only pathogenic function of the polypeptides.[5] It is not known if the proteins perform other functions.[5] If so, it is not known what these hypothetical functions are and how they work.[5] Future research should be aimed to address these possibilities.

Dengue fever is a growing global concern, especially since there are no medicinal treatments. Further research into the polypeptides and their functions could yield highly useful information for drug development.[5]

Chapter 3: Case Study

This case study focuses on Sudeep Kumar, a 36-year-old Indian man who visited his primary care provider and reported flu-like symptoms, including nausea and fever. The purpose of this study will be to analyze Sudeep's signs and symptoms in an effort to ascertain the cause of his illness.

Sudeep Kumar

Chief Complaint

"Since returning to Reno from India, I have been experiencing flu-like symptoms. A rash has also developed on my legs."

History of Present Illness

Sudeep claims that his symptoms began after returning from his summer trip to India. He reports being in good health while in India; however, he was bitten by mosquitoes regularly during the night, since he did not utilize a mosquito net. He claims that aside from being a nuisance, the bites did not seem to result in sickness during his stay, which is why he refrained from acquiring a net. The patient does claim that he utilized repellant, albeit only outdoors.

Sudeep states that his symptoms began about five days after coming home from India. He reports having felt mildly nauseous. To ameliorate his condition, Sudeep decided to stay home from work and rest. He spent most of his time sleeping, occasionally waking up to eat. By the end of the first day at home, he reported feeling worse than before. Sudeep experienced headaches and retro-orbital pain. His nausea caused far greater discomfort, leading him to vomit, albeit without any noticeable blood. He developed rashes on his feet, and also reports extreme

pain in his lower joints. In response to Sudeep's claims of feeling mildly feverish, his wife took his temperature and reported it to be 99°F. Believing he potentially had food poisoning or the flu, Sudeep reasoned that a good night's rest would make a difference by morning. However, his symptoms had only progressed by the following day. Sudeep reported feeling weak and having trouble maintaining balance while upright. He vomited several times more. A subsequent temperature reading by his wife revealed that his temperature had risen to 103.5°F. The pain and rashes in his lower extremity continue to persist and cause Sudeep great discomfort.

Sudeep denies experiencing hemorrhagic symptoms, such as epistaxis, melena, or hematemesis. Sudeep also reports no respiratory tract symptoms, such as cough, sore throat, or nasal congestion.

Social History

Sudeep grew up in Reno, Nevada with his mother, father, brother and sister. After graduating from high school, he attended the University of Nevada, Reno, where he majored in Chemical Engineering. Upon receiving his degree, he managed to find a job in the renewable energy sector. About two years after graduating, Sudeep was wed to his wife. Both he and his wife are very active people, frequenting the gym and ski slopes regularly. Additionally, the two enjoy traveling. Recently, they visited relatives in Madras, India over the summer. About five days prior to his symptoms, Sudeep returned from Madras to his home in Reno. Sudeep and his wife have no children or pets.

The patient denies ever smoking or using drugs, but openly admits to occasional alcohol consumption. Sudeep states that he is not sexually active.

Family History

Sudeep's parents continue to live in Reno; although his brother has moved to Tennessee and his sister has relocated to California. His father is relatively healthy, although he is at high risk for diabetes. Subsequently, he generally avoids eating foods with high sugar content. His mother has high blood pressure. Sudeep's maternal grandfather is still living at the age of 80. Sudeep's brother and sister are both healthy.

Past Medical History

Sudeep has no significant past medical history, and has not been hospitalized.

Medication: Sudeep is currently not taking any prescription or over-the-counter medicine.

Surgical History: The patient underwent surgery for the removal of wisdom teeth when he was 22.

Allergies: No known food or drug allergies.

Immunizations: The patient is up to date on his immunizations.

Physical Exam

Vital Signs:

Height: 5'11" Weight: 150 lbs Blood pressure: 115/70 Temperature: 103.5°F Pulse: 90 beats/minute Respiratory rate: 18 breaths/minute

General:

Appears physically fit, but in clear pain. Alert and oriented.

Skin:

Erythematous maculopapular rash present on legs.[7] Skin is warm to the touch.

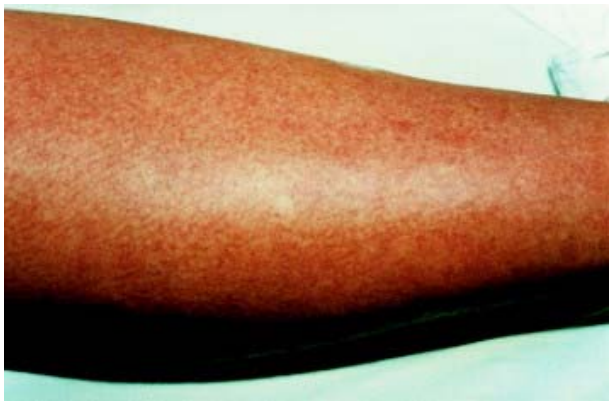

Figure 5: Picture of the maculopapular rash on the patient's legs.[20]

HEENT

Head:

No ethmoid, frontal, or maxillary sinus tenderness. No TMJ tenderness. No lesions.

Eyes:

Pupils are round and reactive to light.

Ears:

External auditory canals are generally clear bilaterally with small amounts of cerumen present. Tympanic membranes are clear and reflex to light bilaterally.

Nose:

No deviation of the septum. No nasal polyps. No discharge.

Throat and Mouth:

Appears normal. Moist mucous membrane, no petechiae or erythema. No oral lesions.

Neck:

Lymph nodes bilaterally swollen. No bruits heard in the carotid arteries. No thyromegaly and no masses.

Chest/Lungs:

No wheezing or rales wile breathing. Appear normal.

Heart:

RRR, heard normal S1 and S2. No m/r/g.

Abdomen:

Appears Normal. Flat and soft. No tender areas. Normal bowel sounds in all quadrants.

Muscularskeleton:

Muscle tone appears normal. Muscle strength is 3/5 in the lower extremities bilaterally but most likely due to pain. Spine is aligned.

Neurological:

Clear speech. Alert and Oriented x 3, No facial droop. Cranial nerves 2-12 are intact. No Focal Deficits.

Diagnostic Work:

Complete Blood Count (CBC)

RBC Count [8]	$4.8\times 10^{6}/\mu$	$(4.7\text{-}6.1\times 10^{6}/\mu)$
Hemoglobin [8]	18 g/dL	(13.8 – 17.2g/dL) male
Hematocrit [8]	51%	(40.7 – 50.3%) male
MCV [8]	84 fL	(80-95fL) male
MCH [8]	29 pg/cell	(27 – 31pg/cell)
White Cell Count [8]	4,200 /µ	(4,500 – 10,000 /µ)
Platelets [8]	148,000 / dL	(150,000 – 450,000 / dL)
ESR [9]	10 mm/hr	(Less than 15 mm/hr) male

Basic Metabolic Panel

BUN [10]	15 mg/dL	7 – 20 mg/dL
Bicarbonate [10]	17 mmol/L	20-29 mmol/L
Creatinine [10]	1.1 mg/dL	0.8 – 1.2 mg/dL
Glucose [10]	75 mg/dL	64 – 100 mg/dL
Chloride [10]	105 mmol/L	101 – 111 mmol/L

Potassium [10]	4.0 mEq/L	3.7 – 5.2 mEq/L
Sodium [10]	138 mEq/L	136 – 144 mEq/L

Cholesterol

Cholesterol [11]	160 mg/dL	(Less than 180-200 mg/dL)
LDL [11]	60 mg/dL	(Less than 190 mg/dL)
HDL [11]	60 mg/dL	(Greater than 40 – 60 mg/dL)

Liver Enzyme Tests

Aspartate aminotransferase (AST) [12]	20 U/L	(10 – 34 U/L)
Alanine Aminotransferase (ALT) [13]	25 U/L	(10 – 40 U/L) male
Alkaline Phosphatiase [14]	70 U/L	(44 – 147 U/L)
Gamma-glutamyltransferase[15]	50 U/L	(8 – 65 U/L)

The tests indicate that the patient is likely suffering from mild plasma leakage. Slightly elevated hematocrit and hemoglobin values suggest decreased fluid levels, leading to increased concentration. Normal values for MCH, MCV, and ESR suggest nothing is wrong with the patient's red blood cells. The patient's RBC count is also within healthy limits, which implies

hemorrhaging isn't a concern. Leukopenia and thrombocytopenia could potentially point towards an immune system deficiency.

The patient's recent trip to India was acknowledged. The patient confirms that all required immunizations were taken. A blood test was ordered to check for the presence of antibodies to any of the various mosquito-borne illnesses, which cannot be vaccinated against. A blood smear and MAC-ELISA were conducted on the patient's serum.[17]

Differential Diagnosis and Supporting Argument

A viral infection was considered. The patient's fever-like symptoms are consistent with the flu. However, the patient denies any respiratory tract issues and confirms that he is up-to-date with his immunizations. The possibility of an influenza infection is unlikely.

Mosquito-borne illnesses were also analyzed. Since Sudeep visited India during the rainy summer months, did not employ mosquito netting while sleeping, only used repellant outdoors, and even suffered multiple mosquito bites, it is highly probable he is suffering from a mosquito virus.

Sudeep's arthalgia, myalgia, and swollen lymph nodes suggest lymphatic filariasis.[18] Classic signs, such lymphoedema and elephantiasis were not present though, so this condition was deemed unlikely.[18]

Malaria was considered on the basis of Sudeep's fever-like symptoms. The presence of a maculopapular rash is not consistent with malaria, however. Malaria would also cause liver strain, which was not indicated by Sudeep's normal liver enzyme tests values. Splenomegaly, a hallmark of the disease, was also not observed. Finally, *Plasmodium* were not detected in the patient's blood smear. Subsequently, malaria was considered with low probability.

Chikungunya and dengue were also considered; although differentiating between the two proved more difficult. Both are transmitted by *Aedes aegypti* and manifest with similar symptoms, such as fever, headache, vomiting, arthralgia and myalgia.[16] Specific blood tests were required. Immunoassays performed on the blood detected the presence of anti-DENV IgM antibodies, and not anti-CHIKV antibodies.[17] Dengue virus was considered the most probable cause of illness.

It was also determined that the patient was most likely suffering from dengue fever (DF), and not the more severe dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS). Although plasma leakage is an indicator of DHF and DSS, Sudeep doesn't seem to be experiencing severe plasma leakage.[19] A review of the HPI and physical exam suggests that Sudeep is not suffering from the hemorrhagic symptoms consistent with DHF or DSS.[19] A review of his RBC further supports this notion. Sudeep likely has DF, which has not yet progressed to the more severe forms.

Treatment

Since illness was detected reasonably early for Sudeep and DHF/DSS were not considered the cause of illness, hospitalization is likely not required. It is advised that Sudeep achieves a high intake of fluid to maintain proper body fluid levels and alleviate the consequences of plasma leakage. For pain management, Sudeep can take acetaminophen, but should avoid aspirin or other anti-inflammatory medication due to increased risk of bleeding. Sudeep's friends and family are in no danger of contracting the illness themselves. Following this regiment, Sudeep should regain his health fairly expeditiously. Should the illness persist or worsen, Sudeep should seek further medical attention and consider hospitalization.

Prognosis and Plan Implementation

Provided Sudeep maintains a steady intake of fluids, he should recover fairly quickly. The patient offers assurance that he will persist with oral hydration as recommended. Until recovery, he will likely continue to experience discomfort from symptoms. If he desires, he can take acetaminophen (a maximum of 4 grams a day) to cope with pain. Sudeep was strongly advised against taking aspirin or other ant-inflammatory medications. The patient confirmed that he would be able to afford medication if desired. Sudeep will likely need to stay at home for his recovery. He claims that missing work will not be a financial burden.

Hospitalization is likely not required, since the illness was detected reasonably early before it could progress to DHF or DSS. Sudeep was advised that mild fever and joint pain would persist for a few days. If these symptoms last for more than a week, he was told to visit an outpatient physician again. If Sudeep begins to experience hemorrhagic symptoms, he was told to go straight to the emergency room of the hospital. Based on the current clinical examination, Sudeep was reassured that his condition is not severe enough to warrant this latter scenario. Provided he maintains proper oral hydration, Sudeep will avoid hospitalization and recover quickly. Sudeep states that, should hospitalization occur, he will likely be able to cover expenses; although, he would prefer to avoid that situation in the first place.

After recovering from DF, Sudeep will be immunized against the particular serotype he was infected with, making reinfection to the specific serotype highly improbable. Sudeep will also not be able to transmit the virus, so it is safe for him to stay with his wife. Although unlikely, since symptoms would have probably manifested by now, Sudeep's wife could also be infected. Sudeep was informed that if his wife showed similar symptomatology, she should go to an outpatient physician.

Sudeep can still be infected with the other serotypes of the dengue virus. Subsequently, he was informed about the spread of mosquito-borne diseases and the precautions needed to avoid a bite. Sudeep was advised that should he return to India in the future, he should use mosquito repellent both indoors and outdoors, employ mosquito netting when sleeping, and wear clothes which cover as much skin as possible.

Bibliography

1. Dengue. (n.d.). Retrieved December 19, 2015, from https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/dengue

2. Dengue Homepage. (2010, July 28). Retrieved November 18, 2015, from http://www.cdc.gov/dengue/epidemiology/

3. Dengue Fever. (n.d.). Retrieved November 18, 2015, from http://www.webmd.com/a-to-z-guides/dengue-fever-reference

4. Dengue and severe dengue. (n.d.). Retrieved November 18, 2015, from http://www.who.int/mediacentre/factsheets/fs117/en/

5. Muñoz-Jordán, J., Sánchez-Burgos, G., Laurent-Rolle, M., & García-Sastre, A. (2003). Inhibition of interferon signaling by dengue virus. *PNAS,100*(24), 14333–14338-14333–14338. doi:10.1073/pnas.2335168100

6. Jones, M., Davidson, A., Hibbert, L., Gruenwald, P., Schlaak, J., Ball, S., Jacobs, M. (2005). Dengue Virus Inhibits Alpha Interferon Signaling by Reducing STAT2 Expression. *Journal of Virology*, *79*(9), 5414–5420. http://doi.org/10.1128/JVI.79.9.5414-5420.2005

7. Gubler DJ. Dengue and Dengue Hemorrhagic Fever. *Clinical Microbiology Reviews*. 1998;11(3):480-496.

8. Chen, Y. (2014, November 26). CBC Blood Test: MedlinePlus Medical Encyclopedia. Retrieved April 4, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003642.htm

9. Martin, L. J. (2015, May 3). ESR: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003638.htm

10. Martin, L. J. (2015, April 30). Basic metabolic panel: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003462.htm

11. Chen, M. A. (2014, November 26). Cholesterol testing and results: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/patientinstructions/000386.htm

12. Martin, L. J. (2015, February 8). Aspartate aminotransferase (AST) blood test: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003472.htm

13. Martin, L. J. (2015, February 8). Alanine transaminase (ALT) blood test: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003473.htm

14. Martin, L. J. (2015, April 30). ALP - blood test: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003470.htm

15. Martin, L. J. (2015, February 8). Gamma-glutamyl transpeptidase (GGT) blood test: MedlinePlus Medical Encyclopedia. Retrieved April 07, 2016, from https://www.nlm.nih.gov/medlineplus/ency/article/003458.htm

16. Sharp, T. M. (2014, September 15). Differentiating Chikungunya From Dengue: A Clinical Challenge. Retrieved April 7, 2016, from http://www.medscape.com/viewarticle/831523#vp_1

17. Dengue Homepage: Laboratory Guidance and Diagnostic Testing. (2016, January 20). Retrieved April 07, 2016, from http://www.cdc.gov/dengue/clinicalLab/laboratory.html

18. Lymphatic filariasis. (n.d.). Retrieved April 24, 2016, from http://www.who.int/mediacentre/factsheets/fs102/en/

19. Clinical/Laboratory Guidance. (2014). Retrieved April 24, 2016, from http://www.cdc.gov/dengue/clinicalLab/clinical.html

20. Solomon, T. (2000). Neurological manifestations of dengue infection. *The Lancet*,*355*(9209), 1053-1059. Retrieved April 24, 2016.