BIOLOGICAL ACTIVITIES OF DENGUE VIRUS ON MURINE MACROPHAGES

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ABSTRACT: The biological activities of dengue 2 virus on its target cells, namely macrophages and neutrophils were investigated. Preliminary results showed that polyethylene glycol precipitated dengue 2 virus acted as a chemoattractant for peritoneal cells of Balbc/J mice and for established monoctyes/macrophage cell lines (WEHI 265.1). This *in vivo* response was also seen *in vitro* using Boyden Chemotaxis chambers and was found to be inhibited by specific viral antibodies. The ability of the virus to activate these cells was then evaluated. Treatment of the peritoneal cells with virus induced a respiratory burst with the production of picomoles of superoxides. In addition, the virus induced production of reactive nitrogen intermediates (1-2 μM) and also resulted in exocytosis of lysozyme. Adhesiveness was also observed and appeared to increase with higher concentrations of the virus. The above results demonstrate that increased adhesiveness and activation are induced following treatment of peritoneal cells and the cell line WEHI 265.1 with dengue virus. *(JUMMEC 1997 2(1): 11-17)*

KEYWORDS: Macrophage, macrophage activation, dengue virus, adhesion

Introduction

Dengue Fever is an acute, infectious febrile disease which is caused by four serotypes of dengue virus (Den 1-4). It has been reported in over 100 countries and poses a threat to approximately 2 million people (1). A more severe form of the disease, Dengue Haemorrhagic Fever (DHF) is characterized by abnormalities of haemostasis and increased vascular permeability, which in some instances results in hypovolemic shock syndrome, Dengue Shock Syndrome (DSS). Dengue viruses are transmitted through the bite of an infected mosquito, namely *Aedes aegypti*, which is found in tropical and subtropical regions of the world (1). Infections in humans with one serotype produces life-long immunity to re-infection by that serotype but only transient protection against other serotypes. Because of the significant morbidity and mortality attributed to this virus, efforts are being made to develop immunogenic vaccines. Epidemiological studies have shown that DHF/DSS is observed more commonly in secondary dengue virus infections than primary infections (2). Hence protective immune responses should induce immunity that will not increase the risk of DHF/DSS in future infections. The pathogenesis of the more severe forms is only partially understood. Several hypotheses have been proposed to explain the pathogenesis of DHF/DSS (3, 4, 5). The most popular of these suggests an immunopathological mechanism involving enhancing antibodies and cell mediated immunity (CMI) during a second dengue infection. During such an infection, with a different dengue serotype, it is envisioned that subneutralzing, 'enhancing' antibodies promote viral entry and replication in monocytes/macrophages (6). These infected cells are then thought to become targets of an immune elimination response, probably mediated by dengue-specific cytotoxic T lymphocytes (CTL), which then results in the release of various mediators which produce the symptoms of DHF/DSS (6). These mediators which increase vascular permeability and the precise mechanism of the bleeding phenomenon seen in dengue are still not known. Another hypothesis presumes that differences in virulence of dengue strains (4) may also contribute to the pathogenesis of DHF/DSS. Changes in the virulence of virus strains could emerge as a result of selective mutation and genetic recombination. In the present study we attempted to further understand the pathogenesis by studying virus-target cell interactions. As the main target cell is the monocyte/macrophage it was decided to investigate the effects of the virus on this cell and to see if any of the functions of the cell were affected. Our results indicate that polyethylene glycol precipitated dengue 2 virus acts as a chemoattractant for Balbc/J and C3H/Hej mouse macrophages both *in vivo* and *in vitro*. We also observed that the virus stimulated macrophages (both activated peritoneal exudates and established cell lines) to release enzymes such as lysozyme and produce nitric

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oxide. Virus infection also induced these cells to adhere to fibrinogen and also resulted in the production of picomole quantities of superoxides.

Materials and Methods

Animals. Six to twenty week old male and female Balb/cJ and C3H/Hej mice were maintained in the Central Animal Facility, University Malaya at room temperature.

Cell lines. The WEHI-265.1 monocytic cell line was obtained from the American Tissue Culture Collection (ATCC, Rockville, MD) and was cultured in RPMI containing 10% Fetal Calf serum.

Reagents. The cytokine, recombinant mouse interferon gamma was purchased from Genzyme Corporation USA. N-formylmethylleucylphenylalanine (FMLP) and phorbol 1,2-myristate 1,3 acetate (PMA) were purchased from Sigma Chemical Co.

Virus. A prototype strain of dengue-2 virus (NGC) was used. The procedure for preparation and titration of virus stocks have been described previously (6). Viral propagation in tissue culture were carried out using the method of Kuno (8). Briefly cell cultures were inoculated with DEN 2 viruses and monitored daily for haemagglutinating activity. Once the titre reached 1:32 or more, the culture medium was collected and virus precipitated with polyethylene glycol and purified through centrifugation on sucrose gradients. Uninfected supernatants were treated similarly.

Isolation of peritoneal cells. Peritoneal cells were isolated as described earlier (9). Briefly, 3-4 mice were injected intraperitoneally with sterile sodium casein in endotoxin-free PBS (autoclaved 1 hr at 20 psi). After 16-24 h, animals were given a second casein injection. Three hours later peritoneal cells were harvested in 3-5 ml RPMI. The cells were then washed and counted.

Chemotaxis assay. *In vivo* Chemotaxis: Four uninoculated mice were sacrificed at the start of the experiment and peritoneal cells harvested in 3-5 ml RPMI medium. They were counted using a hemocytometer, cytospinned onto a slide and then stained with a leucostat stain (Fisher Sc. Co.). 20 mice were inoculated intraperitoneally with 0.3 ml dengue 2-infected C6/36 cell supernatant (5×10^4 pfu/ml) while another 20 mice were inoculated with 0.3 ml of uninfected cell supernatant. After 3, 6, 9 and 24 hours, four mice (both control and virus infected) were sacrificed, peritoneal cells harvested, counted and cytospin preparations carried out.

In vitro Chemotaxis: Cell migration was evaluated by using the 48-well Boyden microchamber (Neuroprobe) (12). Peritoneal cells (4×10^6 cells/ml) were washed and suspended in endotoxin-depleted RPMI with 1% BSA. 50 µl of the indicated cell population (2×10^5 cells) was added to the upper well of the Boyden chamber. 27 µl of solution containing appropriate dilutions of virus/FMLP/PMA (which were also endotoxin depleted) were placed in the lower microchamber wells. In some experiments virus solutions were preincubated with specific antiviral antibody. The wells were separated by a 5 µm pore size polycarbonate filter. All responses were assayed in triplicate. After incubation at 37° C for one and a half hours the filters were scraped to remove non-migrating cells from the upper surface. The filters were subsequently fixed in acetone and stained with a leucocyte stain. The numbers migrating were determined at 400 x magnification and the cells of the indicated lineage were enumerated. Specific chemotaxis represents the average number of migrating cells per 5 high power fields minus the mean number of cells migrating in medium alone. To show specificity of binding, virus was incubated with excess DEN 2 specific monoclonals (3H5-ATCC) at room temperature prior to loading of the wells.

Superoxide production. The production of extracellular superoxide was measured using superoxide dismutase inhibitable reduction of ferricytochrome c (10). Mouse peritoneal cells (2×10^6) were added to the assay mixture containing cytochrome c before addition of the stimulus. Superoxide production was assayed spectrophotometrically (570 nm) as a function of ferricytochrome c reduction. To exclude the presence of other reductants, a reference sample containing 3 µl superoxide dismutase (SOD, 0.8 mg/ml, Sigma Chemical Co.), an enzyme that catalyzes the conversion of superoxide to hydrogen peroxide and oxygen was included as a control for each sample. The rate of cytochrome c reduction is subtracted from that in the samples. The resulting value equals the rate of SOD-inhibitable cytochrome c reduction. 10 ml of purified peritoneal cells (20×10^6/ml) in HBSS containing Ca^{2+} and Mg^{2+} (Sigma Chemical Co.) were incubated at room temperature with 187 µl ferricytochrome c (1.3 mg/ml in HBSS, Sigma Chemical Co.) and 1-5 µl of PMA (1 mg/ml) or 5-10 µl dengue 2 virus (5×10^4 pfu/ml) in 96-well microtitre plates. The amount of superoxide produced was calculated and expressed as pmol cytochrome c reduced/min/10^6 cells using the formula:

$$\frac{\Delta A/\text{min} \times \text{reaction volume} \times 10^6 \text{ nmol/mmol} \times 1000}{(\text{Cy/mmol}) \times 1000 \text{ ml/l}}$$

where ΔA/min = change in absorbance per minute and Cy = extinction coefficient of cytochrome c, which is 21.1 mm/cm.

Measurement of Lysozyme release. Casein-elicited peritoneal cells (5×10^5) suspended in PBS containing 0.3% BSA were incubated with various virus dilutions at 37° C for 2 hours. The reaction was stopped

by rapid cooling on ice followed by centrifugation. Lysozyme activity was then determined from the supernatants by a turbidometric method as described previously (9, 13). Briefly, 50 µl of supernatant was added to 150 µl of the substrate, which consisted of 30 mg/ml *Micrococcus lysodeikticus* (Sigma Chemical Co.) in 50 nM phosphate acetate buffer, pH 6.0 and 0.05% Triton X (BioRad). Chicken egg-white lysozyme (Sigma Chemical Co.) was used as a standard. The activity was measured at an optical density of 540 nm after 20-30 minutes.

Nitrate assay. Peritoneal cells (5 X 10^5) were cultured for 2 hours in RPMI containing 10% FCS for 2 h at 37° C before use. The medium was replaced with 100 ml of fresh serum medium and the cells were incubated with dengue virus or γ-IFN for 24 h at 37° C. WEHI 265.1 cells were similarly treated. The nitrite concentration of the 24 h conditioned medium was measured by a microplate assay as described by Ding *et al.* (11). Briefly 90 µl of conditioned medium was incubated with 90 µl of Greiss reagent (1% sulfanilimide, 0.1% naphthyethylene diamine dihydrochloride (Sigma Chemical Co) and 2.5% H_3PO_4, (Fisher-Scientific, Pittsburgh, PA) at room temperature for 10 mins. The absorbance at 570 nm was determined in an ELISA plate reader. Cell free medium alone contained 0.03 µM nitrite per well, hence this value was determined in each experiment and subtracted from the values obtained with the cells. Dilutions of a 1 µM stock solution of sodium nitrite (Sigma Chemical Co.) were used to obtain a standard curve. To show specificity of binding, virus was incubated with excess dengue 2 specific monoclonals (3H5-ATCC) at room temperature prior to loading of wells.

Adhesion assay. The method of Devi *et al.* (9) was used. Briefly, ninety six flat-well bottom plates were coated with 25 µl of purified fibrinogen (2 mg/ml in PBS, R & D systems) and incubated for 90 minutes at room temperature. Plates were then washed 3 times in HBSS containing 10 mM HEPES, pH 7.3, 1 mM $MgCl_2$, and 0.5% BSA. Finally the stimuli (10^3 -10^5 pfu dengue virus or 10 ng/ml PMA) were added followed by addition of target cells (5 X 10^5 cells/well) and incubated for 20 to 30 minutes at room temperature. Wells were then gently washed with the same buffer. 200 µl of the buffer without BSA was added, followed by 10 µl of the vital dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; thiazolyle blue MTT (Sigma Chemical Co). This mixture was incubated 4-16 hours at 37° C. The supernatant was very carefully aspirated and 100 µl of acid isopropanol (2 mM HCl) was added to the cells, vortexed, and the optical density read at 540 nm. To generate a standard curve, wells containing graded numbers of cells were also stained with the dye. To show specificity of binding, virus was incubated with excess DEN 2 specific monoclonals (3H5-ATCC) at room temperature prior to loading of wells.

Results

Chemotaxis. The intraperitoneal inoculation of dengue virus resulted in an influx of cells into the peritoneal cavity within 3 hours (Table IA). In the controls, there was only a relatively small increase in the numbers of infiltrating cells which steadied after 9 hours. This was probably a normal immune response to foreign material injected into the peritoneal cavity. Cells were mainly macrophages (97.9%), some neutrophils (2%) and few lymphocytes (Table IB). The increase in macrophages was more than tenfold. By 3 hours the increase was found to be significant. These increases peaked at 9 hours post infection suggesting recruitment of inflammatory cells to the site of infection (Table IA). This *in vivo* response was seen *in vitro* using the Boyden Chemotaxis chambers (Table II). Treated uninfected tissue culture supernatant was used as the negative control and FMLP as the positive control. With FMLP, cells migrated across the membrane with concentrations as low as 10 ng/ml and as the concentration of

Table IA. Kinetics of the inflammatory response induced by intraperitoneal injection of dengue virus

Stimulus	Total cell count (X 10^6 cells/ml)#			
	3 hrs	6 hrs	9 hrs	24 hrs
Control S/N	0.3±0.12	2.89±1.15	0.51±0.29	0.31±0.01
Virus*	1.24±0.28	2.54±0.20	9.91±1.09	4.83±0.57

Mice were inoculated with treated infected (Virus) or uninfected (Control S/N) tissue culture supernatant intraperitoneally and peritoneal cells harvested as indicated. All values corrected for zero time reading.
# $p<0.05$ (values are an average of 3-5 experiments)
*5 x 10^4 pfu/ml

Table IB. Composition of exudate induced by intraperitoneal injection of dengue virus

Composition of exudate	Stimulus							
	Nil				Virus			
	3 hrs	6 hrs	9 hrs	24 hrs	3 hrs	6 hrs	9 hrs	24 hrs
% Macrophages	87.6	97.8	87.4	ND	89.9	97.9	97.0	93.1
% Neutrophils	12.4	2.1	12.6	ND	9.9	2.03	2.59	6.9
% Lymphocytes	0	0	0	ND	0	0.043	0.39	0

Cytospin preparations were stained with a leucostat stain and an average of 5 fields were determined. (Values are an average of 3 experiments)
ND - not done

Table II. *In vitro* Chemotaxis

Stimulus	Dose	Cells migrated (10^4 cells/ml)
Control S/N	-	0
FMLP	10 ng/ml	0.054 ± 0.008
	100 ng/ml	12 ± 0.200
	1000ng/ml	30 ± 0.400
Dengue 2 virus	5×10^3 pfu/ml	0.37 ± 0.004
	5×10^4 pfu/ml	1.3 ± 0.040
	5×10^5 pfu/ml	9.7 ± 1.800
Dengue 2 virus + 3H5*	5×10^3 pfu/ml	0.042
	5×10^4 pfu/ml	0.042
	5×10^5 pfu/ml	0.054

Peritoneal cells were assayed in Boyden microchambers. The number of cells per 5 high power fields was determined. Background migration in medium alone was subtracted from the data presented here.
*3H5 -Dengue 2 specific monoclonals were incubated with the virus for 30 minutes prior to loading of wells (1 experiment)

FMLP - N-formylmethylleucylphenylalanine

FMLP increased so did the concentration of the cells that migrated across the membrane. Dengue 2 virus also stimulated migration of the cells but to lesser degree. In preliminary experiments, varying doses (5×10^3 - 5×10^5 pfu/ml) of dengue 2 virus were used to stimulate migration of cells. As little as 5×10^3 pfu/ml of virus stimulated migration. To establish specificity *in vitro* neutralization experiments with monoclonal antibody to dengue virus were performed. Migration was inhibited when the virus was incubated with dengue-2 specific monoclonals prior to loading. This could either be due to a neutralization effect or a blocking effect, hence needed appropriate signals for the migration are not released. Control mouse monoclonals had no effect when used at the same concentrations as the dengue specific monoclonals (data not shown).

Induction of a respiratory burst. During the respiratory burst that accompanies the activation of phagocytic cells, high levels of reactive oxygen products are released (9). Phagocytic cells produce two major reactive oxygen species namely, superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2). Superoxide is produced by a NADPH-dependent oxidase enzyme that is activated upon cellular stimulation. When neutrophils and other phagocytic cells ingest opsonized particles, a rapid respiratory burst ensues, resulting in the formation of O_2^-, H_2O_2, and hydroxyl radicals. In preliminary experiments varying doses of dengue virus, PMA (positive control) and treated uninfected tissue culture supernatant (negative control) were used to stimulate the neutrophils and macrophages. Preliminary experiments showed that as little as 0.001 ng/ml of PMA was sufficient to stimulate peritoneal cells to produce a respiratory burst. The minimum viral dose necessary to stimulate measurable respiratory activity was 5×10^4 pfu/ml (Table III).

Table III. Dengue virus stimulates peritoneal cells to generate superoxides

Stimulus	Dose	pmole Cyt c reduced
Control S/N	-	0
PMA	0.01 ng/ml	2.13 ± 0.13
	0.05 ng/ml	5.2 ± 0.68
Virus	5×10^5 pfu/ml	7.9 ± 2.60

Casein elicited peritoneal cells were treated with Control S/N/Virus/PMA. After 30 minutes cultures were assayed for extracellular O_2^- release as stated in the materials and methods. The data presented represent an average of 5 experiments.

Granule exocytosis. The proteolytic enzymes stored within the granules of phagocytic cells are involved in bactericidal and digestive functions (14). When neutrophils are exposed to stimuli (e.g., IL-8), degranulation results in the release of several potent proteolytic enzymes as well as bactericidal enzymes. Lysozyme, a small cationic enzyme, is found in the secondary granules and is also present in azurogranules (9). Lysozyme is bactericidal for some bacteria and its primary function is to digest cellular debris. As before, PMA was the positive control. The ability of dengue virus to induce release of lysozyme is shown (Table IV), the minimum dose being 5×10^4 pfu/ml as this concentration was found necessary for detectable levels of granule exocytosis.

Table IV. Dengue virus stimulates granule exocytosis

Stimulus	Dose	Lysozyme (ng/ml)
Control S/N	-	0
PMA	1 ng/ml	119 ± 37.40
Virus	5×10^5 pfu/ml	111.5 ± 44.5

Cells were incubated with the stimuli and 2 hours later the culture supernatant was assayed for lysozyme activity. Data presented represent an average of 5 experiments.

Production of nitrite. Reactive nitrogen intermediates (RNI) express a high degree of chemical activity. RNI are secretary products of activated macrophages but are also secreted by other cell types. The nitrogen intermediates are derived from nitric oxide

(NO) and include nitrite and nitrate. NO has been identified as a pleiotropic intercellular messenger that activates guanylate cyclase, leading to an increase in cGMP accumulation (15). NO is responsible for regulating a variety of diverse cellular functions in many tissues (16). In the following experiments we examined the ability of dengue virus to stimulate RNI activity measured by the production of nitrite. As can be seen in Table V dengue virus induced significant amounts of nitrite by both peritoneal cells and the monocytic tumor cell line WEHI-265.1. Comparable levels of nitrite were obtained between the positive controls (PMA and γ-IFN) and 5 X 10^4 pfu/ml of virus.

Table V. Production of reactive nitrogen intermediates by dengue 2 virus activated cells

Cells	Stimulus	Dose	Nitrite (μM)
WEHI 265.1	Control S/N	-	0
	PMA	0.01 ng/ml	2.004 ± 0.161
		0.05 ng/ml	4.696 ± 1.521
	Virus	5 x 10^3 pfu/ml	0.904 ± 0.052
		5 x 10^4 pfu/ml	2.207 ± 0.448
Peritoneal	Control S/N	-	0
Cells	γ-IFN	10 ng/ml	1.378 ± 0.487
	Virus	5 x 10^4 pfu/ml	0.791 ± 0.032
		5 x 10^5 pfu/ml	0.860 ± 0.003
	Virus + 3H5*	5 x 10^4 pfu/ml	0.0001

Caesin elicited peritoneal cells or WEHI-265.1 monocytic tumor cells were cultured with indicated virus or γ-IFN. Conditioned medium wasassayed for production of reactive nitrogen intermediates 24 hours later. Data represent the average of 5 experiments.

*Dengue specific monoclonals were incubated with the virus prior to incubation.

Adhesion to fibrinogen. The integrin CR3(CD11b/18) acts as a receptor for fibrinogen (17, 18). Modulation of β-integrins can be monitored by changes in adhesiveness to fibrinogen. To determine whether dengue virus stimulation contributed to the adhesiveness of inflammatory cells, we evaluated this effect of dengue 2 virus on peritoneal cells and WEHI-265.1 monocytic tumour cells. It was observed that viral stimulation induced binding of cells to fibrinogen and as the number of plaque forming units/ml increased so did the number of cells adhering to fibrinogen (Table VI). As little as 5 X 10^3 pfu/ml of virus stimulated adhesion. This effect was abolished when the virus was preincubated with dengue 2 specific monoclonal antibodies (3H5-1).

Table VI. Dengue virus induces adhesiveness to fibrinogen

Stimulus	Dose	No of cells adherent (x 10^5 cells/ml)
Control S/N	-	0
PMLP	10 ng/ml	56 ± 26.0
Virus	5 x 10^3 pfu/ml	1.7 ± 0.1
	5 x 10^4 pfu/ml	4.3 ± 0.2
	5 x 10^5 pfu/ml	182.5 ± 5.5
Virus + 3H5*	5 x 10^5 pfu/ml	0.001

Casein elicited peritoneal cells were added to plates which had been coated with fibrinogen and to which appropriate amounts of stimulants had been added. Non-specific binding in the absence of stimulants was subtracted from the data. Data presented represent an average of 5 experiments.

*Dengue 2 specific monoclonals were incubated with the virus for 30 minutes prior to loading of wells.

Discussion

Dengue virus infections have become a major problem and the global increase in DHF/DSS is of grave concern to many countries around the world. Attempts to prevent the spread of the disease by vector control has had at best limited success. Vaccination is the most cost effective way to prevent DF and DHF/DSS. A tetravalent live vaccine is already undergoing field trails with promising results (1). The pathogenesis of the disease is only partially understood and is said to involve enhancing antibodies and cell mediated immunity especially during a secondary infection. As the main target cell is the macrophage we have attempted to further understand the pathogenesis by studying virus-target cell interactions. It was found that when virus was injected intraperitoneally into mice there was a rapid influx of cells, mostly macrophages, into the peritoneal cavity. Here dengue virus appeared to act as a chemoattractant for macrophages. It was also seen that peritoneal macrophages tend to aggregate or clump together after virus treatment. Furthermore, these *in vivo* responses were demonstrated *in vitro* using the Boyden Chemotaxis chambers. Specificity of the migration was established when this movement was inhibited by specific antibodies to the virus. Macrophage functions tests were then carried to determine to see if the virus directly activates its target cell. From the superoxide assays, it is clearly seen that dengue virus induced a respiratory burst with the production of superoxide anion in macrophages and neutrophils. In addition dengue virus induced granule exocytosis of lysozyme in the same population of cells. Significant amounts of lysozyme was released. Lysozyme is mainly involved in digesting cellular material and foreign bodies.

Reactive nitrogen intermediates are another group of compounds which have a high degree of chemical activ-

ity. The reactive nitrogen intermediates include nitrite and the related highly reactive oxides such as nitric oxide (NO) and nitrogen dioxide. NO production by rodent macrophages is clearly involved in the nonspecific host defence system against foreign pathogens and tumour cells (16). Dengue virus was found to induce mouse peritoneal cells and the monocytic tumor cell line WEHI 265.1 to produce significant amounts of NO/nitirite (1-2 μM NO_2^-).

Another important preresquisite for migration of leucocyte from the circulation to an inflammatory site is increased adhesion to matrix proteins and endothelium. In our study it is seen that dengue 2 virus stimulates casein-elicited peritoneal cells and the monocytic tumor cell line WEHI-265.1 to adhere to fibrinogen.

The present studies indicate that adhesiveness and increased activation (as observed by respiratory burst, production of lysozymes) occurred following treatment with dengue 2 virus. Each of these activities must be coordinated to direct phagocytic cells from the blood stream into tissue spaces where these cells generally exercise their phagocytic and proinflammatory activities. The strategy to affect a coordinated inflammatory response involves varying sensitivities of the target cell populations to different concentrations of virus (10^4-10^5pfu/ml). As seen in the above study adhesion required the lowest concentrations (5×10^3 pfu/ml) of the virus. Migratory activity is seen with at least 5×10^4 pfu/ml of virus with maximal activity at tenfold higher concentrations. However target cell activation (exocytosis, respiratory burst and NO release) is observed at best with concentrations of at least 5×10^5 pfu/ml of virus. Hence only at the focus of infection where the concentration of the virus is the highest are the phagocytic functions activated. Premature activation could be deleterious to normal tissues hence this stepwise gradient serves to focus the site of infection.

The pathogenic mechanisms in dengue virus infections are difficult to elucidate because of the absence of a suitable animal model. Antibody enhancement has been suggested as a possible contributing factor with the main target cell being a monocyte/macrophage. Considerable doubt remains as to the identity of the receptors involved in the uptake of dengue virus by the macrophage/monocyte. The adherence of infected monocytes may be sufficient to trigger off viral release and other cell contents, including factors affecting vascular permeability (19). Blood monocytes are a very heterogenous population of cells, most probably as a result of some differentiation in the 36-104 hours that they spend in blood circulation (20). Hence different stages of differentiation of this population of cells may have varying susceptibilities to the virus. Viral replication is said to be enhanced in the presence of low levels of non-neutralising antibodies (3). In the absence of antibodies very little replication has been demonstrated (19,21). This could imply effective clearance. However enhanced cytolysis of cells by viral infections are observed following infection in the presence of antibody. Thus the interactions seen in the above studies suggest clearance in the absence of antibody most probably in a primary infection and within 24-48 hours as most of the assays carried out were done within 24 hours. Also the absence of activity/detection seen in the presence of antibody could imply neutralization by antibody or enhanced replication. Further work is being done to determine macrophage functions in the presence of antibody for longer periods of infections.

Acknowledgements

The work was supported by the University of Malaya R&D fund 3-07-04-504.

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