Solar inactivated *Vibrio cholerae* induces maturation of JAWS II dendritic cell line *in vitro*

Cornelius Cano Ssemakalu, Eunice Ubomba-Jaswa, Keolebogile Shirley Caroline Mamotswere Motaung and Michael Pillay

ABSTRACT

Solar disinfection (SODIS) has been shown to reduce the risk associated with the contraction of water borne diseases such as cholera. However, little or no research has been undertaken in exploring the role played by the immune system following the consumption of solar inactivated water pathogens. This study investigated the potential for solar inactivated *Vibrio cholerae* to induce the maturation of dendritic cells *in vitro*. Dendritic cells are professional antigen presenting cells found in mammals. However, only in their mature form are dendritic cells able to play their role towards a long lasting immune response. Three strains of *V. cholerae* were solar irradiated for 7 hours. Thereafter, the solar irradiated, non-solar irradiated, phosphate buffered saline prepared and heat/chemically inactivated cultures of *V. cholerae* as well as lipopolysaccharide and cholerae toxin-β subunit were used to stimulate immature dendritic cells. After 48 hours, the dendritic cells were assessed for the expression of CD54, CD80, CD83, CD86, MHC-I and MHC-II cell surface markers. Results show that solar inactivated *V. cholerae* was able to induce maturation of the dendritic cells *in vitro*. These findings suggest that there may be an immunological benefit in consuming SODIS treated water.

Key words | cholera, disinfection, immunity, SODIS, vaccine, waterborne

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HIGHLIGHTS

- Seven hours of solar irradiation sufficiently inactivates the culturability of toxigenic and non-toxigenic strains of *V. cholerae*.
- Solar inactivated *V. cholerae* lose the ability to infect and also kill dendritic cells in vitro.
- Solar inactivated *V. cholerae* induces the phenotypic maturation of immature dendritic cells in vitro and thus could enable antigen presentation.

INTRODUCTION

The spread of water-borne diseases through the consumption of microbiologically contaminated water still remains a major problem in low income countries (Rosellini & Pimple 2010; WHO 2011). This is attributed to the inability of these countries to effectively and efficiently protect their water sources from faecal contamination. The presence of faecal material in natural water resources used by local communities has often resulted in unmanageable outbreaks of waterborne diseases such as cholera (Rosellini & Pimple 2010; WHO 2011). Cholera is a life threatening infection that claims many lives in East Asia, South America and the Sub-Saharan African region yearly (WHO 2014). This infection is caused by pathogenic members of the species of a Gram-negative microorganism known as *Vibrio cholerae* which has been reported to exist naturally within aquatic environments (Merrell *et al.* 2000). Measures such as proper sanitation and hygienic practice as well as poor infrastructure development or improvement have been

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suggested to mitigate the spread of cholera. However, the cost of implementing these measures as well as the technicalities involved remains a major challenge within developing countries.

Solar Ultraviolet Radiation (SUVR) has been proposed as a means of treating microbiologically contaminated water in areas with hot and sunny climates through a process known as SODIS (Conroy *et al.* 1996, 1999; Smith *et al.* 2000; Du Preez *et al.* 2010). The consumption of SODIS water in water-borne disease endemic areas such as those found in the Sub-Saharan African region, and various East Asian countries, has enabled a reduction in the percentage of individuals acquiring water borne diseases (Conroy *et al.* 1996, 2001; Du Preez *et al.* 2010). This observation has mainly been attributed to the ability of SUVR to inactivate vital microbial cellular process (Bosshard *et al.* 2010a, 2010b) as well as compliance by the users (Conroy *et al.* 1999, 2001; Du Preez *et al.* 2010).

Currently, very little is known about the influence that SODIS water consumption may have on the immunity of its consumers (Ssemakalu *et al.* 2014). It is well known that microbial intracellular and cell membrane components of microorganisms are immunogenic (Laflamme *et al.* 2001; Moon 2003). However, the extent of their immunogenicity is dependent on the conditions under which the antigens were generated. Although bacterial cellular components generated following SODIS of water borne microorganisms may be antigenic there is no evidence to show the extent of their potential to elicit an immune response. Therefore, this study set out to determine the effect that SODIS of water containing *V. cholerae* may have on the phenotypic development of dendritic cells *in vitro*.

Dendritic cells are a family of professional Antigen Presenting Cells (APC) that are uniquely distributed within the peripheral tissues, lymph and secondary lymphoid organs and accumulating in regions where macrophages and B cells are generally excluded. These cells play a crucial role in the immune system which is marked by their ability to bridge the gap between the innate and the adaptive immune systems (Trombetta & Mellman 2005). Dendritic cells need to undergo a maturation process to perform optimally. Mature dendritic cells promote efficient and specific immune responses as opposed to immature ones. Immature dendritic cells are associated with immune unresponsiveness and antigenic tolerance (Lavelle *et al.* 2003; Rey-Ladino *et al.* 2005).

The maturity of dendritic cells has been associated with the up-regulation and fundamental distribution of the major histocompatibility classes (MHC) I and II; expression of co-stimulatory molecules such as CD40, CD54, CD80, CD83 and CD86; and an increase in antigen processing capacity and marked rearrangement of adhesion molecules that are likely to allow dendritic cell migration to lymphoid organs (Lavelle *et al.* 2003; Muriel 2003).

An immortalized dendritic cell line known as JAWS II that was established from the bone marrow cultures of $p53^{-/-}$ C57BL/6 mice (Mackay & Moore 1997) was used to achieve the aim of this study. This cell line has been used in antitumor and pathogen specific immunity studies (Jiang *et al.* 2008). Furthermore, it has also been shown to display similar maturation characteristics such as those exhibited by bone marrow derived dendritic cells (BMDCs) (Jiang *et al.* 2008; Egger *et al.* 2011). The use of this cell line, as opposed to murine BMDCs, negates the need to kill numerous mice for their bone marrow and ensures reproducible results that could be interpreted easily (Jiang *et al.* 2008).

MATERIALS AND METHODS

Cell culture reagents and antibodies

Iscove's Modified Dulbecco's Medium (IMDM), and sterile 1x Phosphate Buffer Saline (PBS) were purchased from Life Technologies (Carlsbad, CA); the antibiotics penicillin and streptomycin were purchased from BioWhittaker (Walkersville, MD) whereas gentamicin was purchased from Melford (Chelsworth, United Kingdom); Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) was purchased from Abcam (Cambridge, United Kingdom); Fetal Bovine Serum (FBS) was purchased from Thermo Scientific (Waltham, MA); the rough form lipopolysaccharide (LPS) from *E. coli* serotype J5 and the cholera toxin beta-subunit (CTB) were purchased from ENZO Life Sciences (Farmingdale, NY); 2-mercaptoethanol (2-ME) and 0.25% Trypsin-0.02% EDTA were purchased from Sigma (St. Louis, MO). All antibodies used for fluorescence-activated cell sorting (FACS) were obtained from Biolegend (San Diego, CA).

Dendritic cell culture

The GM-CSF dependent dendritic cell line also known as the JAWS II cell line was obtained from the American Type Culture Collection (CRL-11904; ATCC Manassas, VA). Cells were grown in a Carbon Dioxide (CO_2) incubator (Thermo Scientific, Waltham, MA), at 37°C with 5% CO_2 in complete culture medium consisting of IMDM supplemented with 10% FBS, 10 U/ml penicillin and 100 μg/ml streptomycin, 0.5 mM 2-ME and 5 ng/ml murine GM-CSF. The medium was pre-incubated at 37°C for at least 15 min to allow it to reach the desirable pH (7.0–7.6) prior to the addition of the cells. The cell cultures were maintained by transferring the non-adherent cells into a centrifuge tube. The remaining adherent cells were washed with 1x PBS to remove any traces of FBS and then treated with a solution consisting of 0.25% trypsin and 0.02% EDTA at 37°C for 2 min. The non-adherent and trypsinised cell suspensions were then pooled together in a single centrifuge tube, centrifuged at 1,000 rpm for 10 min and the supernatant was discarded. The cell pellet was washed with 1x PBS and resuspended in fresh complete culture medium at the desired cell density.

Vibrio cholerae strains and culture conditions

Three previously characterised strains of *V. cholerae* (Ssemakalu *et al.* 2013) were used in this study: G4222 (toxigenic) was isolated from a cholera patient in Gauteng, South Africa; BRITS01 (toxigenic) was isolated from Brits, South Africa and ENV1009 (non-toxigenic) was isolated at Rand Water (Vereeniging) in South Africa. All strains were stored at −80°C as bacterial stocks on beads. Each strain was revived through culturing on LB agar at 37°C for 18 hours. Then two to five colonies of each isolate were inoculated in LB broth and incubated at 37°C overnight until the stationary phase was reached (Ssemakalu 2011). Stationary phase cultures were used due to their resilience (Berney *et al.* 2006).

Preparation of PBS prepared and inactivated *V. cholerae*

Vibrio cholerae was harvested by centrifugation (3,000 rpm for 10 min) from overnight stationary phase batch cultures grown in Luria Bertani (LB) broth. The pellet for each strain was washed three times with 1x PBS to remove all traces of LB broth. The microorganisms were then diluted in 1x PBS to an optical density ($OD_{600\ nm}$) of 0.01 that corresponded to approximately 7 log CFU/ml. The bacterial strains were inactivated in 1x PBS containing 1.5% formalin at 65 °C for 1 h. The viability for each strain after treatment was assessed using the Miles and Misera drop counting technique (Miles *et al.* 1938). The microorganisms were stored in PBS at 4°C until required.

Preparation of *V. cholerae* for solar irradiation and dark experiments

Vibrio cholerae was harvested by centrifugation (3,000 rpm for 10 min) from overnight stationary phase batch cultures grown in LB broth. The pellet for each strain was washed three times with 1x PBS to remove all traces of LB. The bacteria were diluted in commercially available still bottled water (Bonaqua, Pretoria, South Africa) to an optical density ($OD_{600\ nm}$) of 0.01 before SUVR exposure.

Solar irradiation of *V. cholerae*

Exactly 15 ml of the bacterial suspension was transferred into transparent 25 cm^2 polystyrene unventilated tissue culture flasks. The flasks were exposed to natural sunlight by placing them horizontally on the roof of a building at an elevation of 1,400 m in Pretoria, South Africa (25°44′50.40″S, 28°16′50.50″E) and the ambient atmospheric temperature was recorded hourly for the entire period of exposure. Control samples were prepared in a similar manner, placed next to the experimental ones but were covered with an opaque box to protect them from direct sunlight. Samples were exposed to SUVR in summer during clear sunny days from 0900H to 1630H (7 h). This exposure time was chosen because it is the optimal recommended time for SODIS (Smith *et al.* 2000; Regula & Martin 2002). Following exposure to SUVR, the bacterial samples were prepared for enumeration by the plate count method as well as co-incubation with the dendritic cells.

Enumeration of *V. cholerae*

All bacterial samples were serially diluted in sterile 1x PBS and plated on LB agar using a modified version of the Miles *et al.* (1938) drop counting technique. Briefly, 20 µl of the appropriate dilution was dropped onto sterile nutrient agar plates in duplicate. The plates were incubated at 37°C overnight and only those plates with fewer than 50 discrete colonies per drop were selected for counting. The total count was divided by the number of drops and multiplied by 50 to convert to 1 ml, and then divided by the dilution factor to give the number of CFU/ml.

Stimulation of the JAWS II dendritic cell line with *V. cholerae*

The established JAWS II DC line was incubated with *V. cholerae* cells that had been: (i) prepared in PBS; (ii) inactivated by heat and 1.5% formalin; (iii) solar radiated in water; (iv) non-solar irradiated in water; (v) LPS (1 µg/ml) and (vi) CTB (1 µg/ml). The untreated dendritic cells were used as negative controls while LPS (1 µg/ml) was used as a positive control. Dendritic cells were co-incubated with *V. cholerae* at a multiplicity of infection of 10. Stimulation of the JAWS II dendritic cell line was initially done in complete growth medium without antibiotics and incubated at 37°C in a 5% CO_2 humidified incubator. Antibiotics including gentamicin at 100 µg/ml were added after 4 h of incubation and the experiments were allowed to proceed for another 44 h. The antibiotics were added to prevent the over growth of bacteria in samples in which viable cultures of *V. cholerae* were used.

Flow cytometry analysis

Following 48 h of incubation, the phenotypic analysis of the dendritic cells was performed on a MACSQuant Analyser (Miltenyi Biotec, Bergisch Gladbach, Germany). Prior to flow cytometric analysis, the cells were washed with a cell buffer (2%FBS in PBS), blocked with anti-mouse CD16/CD32 (clone 93) and placed on ice for 15 minutes, followed by washing and dispensing 5×10^5 cells in 100 µl of cell buffer containing fluorophore conjugated antibodies. The cells were then stained with a panel of seven antibodies following the manufacturer's recommended concentration on ice for 30 min. These antibodies consisted of PE/Cy7 conjugated anti-mouse CD40 (clone 3/23), PE-conjugated anti-mouse CD54 (clone YN1/1.7.4), Pacific blue conjugated anti-mouse CD80 (clone 16-10A1), FITC-conjugated anti-mouse CD83 (clone Michel-19), ACP/Cy7 conjugated anti-mouse CD86 (clone GL-1), Alexa Flour 647 conjugated anti-mouse H-2Db (clone KH95) and PerCP conjugated anti-mouse I-A/I-E (clone M5/114.15.2). After staining, the cells were washed twice, resuspended in 0.3 ml of FACS buffer, and then subjected to flow cytometric analysis in which 10,000 events were acquired for each setup. The resultant Flow Cytometry Standard (FCS) files were analysed using FlowJo software X 10.0.7 (Treestar, OR). Positive staining was determined based on a biological comparison control consisting of stained untreated dendritic cells (Maecker & Trotter 2006). The viability of the cells was assessed by trypan blue exclusion on a TC-20 cell counter (Bio-Rad, Hercules, CA) prior to the flow cytometric analysis.

Statistical analysis

All experiments consisted of at least three independent biological replicates and the data were expressed as means ± standard errors. To define statistically significant differences, the data were analysed with one way analysis of variance ANOVA assuming equal variances at a $P < 0.05$ level.

RESULTS

Exposure of *V. cholerae* to solar ultra-violet radiation

All the experiments were carried out in February 2014 during the South African summer on sunny days with clear sky conditions. The water in which the bacterial cultures were irradiated had an average minimum and maximum temperature of 31.2°C (SEM, ±2.4) and 37.97°C (SEM, ±2.4), respectively, on the different days of exposure. After 2 h of solar exposure, all the solar irradiated cultures of the *V. cholerae* strains used were non-culturable (Figure 1).

However, there was no observable loss in culturability of the control samples throughout the entire duration of the

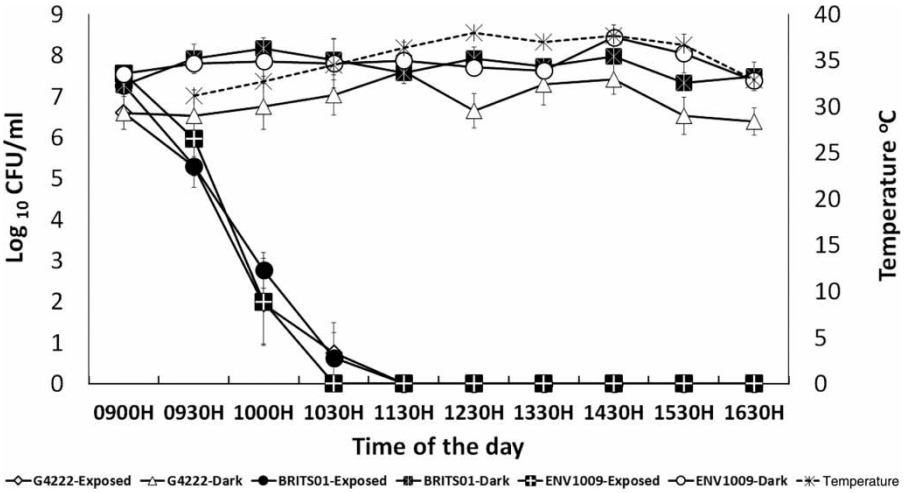

Figure 1 | Solar irradaition of *V. cholerae* in water. Log CFU/mL counts of solar irradiated (Exposed) and non-solar irradiated (Dark) strains of *V. cholerae*. Solar irradiation experiments started at started at 0930H and ended at 1630H. The error bars indicate the mean standard error of independent experiments done on three different days during the South African summer.

exposure. The loss of culturability in the solar exposed samples resulted from the irreversible damage to the microbial cell due to the ultraviolet component of solar radiation and the bacterial cells (Bosshard *et al.* 2010a, 2010b).

Stimulation of immature dendritic cells with *V. cholerae*

Following the stimulation experiments, the viability of the dendritic cells was assessed using the trypan blue method. The results showed that there was a significant loss in the viability of the dendritic cells when stimulated with non-solar irradiated *V. cholerae* cultures (Table 1). A similar result was observed when the dendritic cells were co-stimulated with the heat and chemically inactivated *V. cholerae* strain ENV1009. There was no significant loss in the viability in the dendritic cells that were co-stimulated with solar irradiated *V. cholerae*.

Expression of maturation markers on dendritic cells

Following the co-stimulation experiments, the level of expression of the maturation markers on the dendritic cells was assessed using a 7 colour flow cytometry panel. The maturation markers that were assessed in this study included CD40, CD54, CD80, CD86, MHC-I and MHC-II (Table 2). Prior to the flow cytometric assays, the dyes to which the maturation markers where attached were compensated for against each other to minimise the dye overlap according to the Miltenyi MACSQuant Analyser recommendations. With the correct compensation settings, it was possible to have accurate and precise flow cytometric

Table 1 | Viability of the dendritic cells after the co-stimulation experiments

Dendritic Cell Treatment	Mean (%)	SEM (±)
Untreated	99.00	0.00
LPS (1 μg/ml)	98.33	0.67
CTB (1 μg/ml)	98.33	0.33
G4222 – In PBS	97.67	0.33
G4222 – Inactivated	98.67	0.67
G4222 – Exposed	95.67	1.67
G4222 – Dark	63.00[a]	3.79
BRITS01 – In PBS	97.33	0.88
BRITS01 – Inactivated	91.67	4.41
BRITS01 – Exposed	97.33	0.33
BRITS01 – Dark	83.67[a]	1.86
ENV1009 – In PBS	93.00	3.79
ENV1009 – Inactivated	53.33[a]	1.86
ENV1009 – Exposed	98.00	0.00
ENV1009 – Dark	63.33[a]	6.64

[a]Indicates means that had a $P < 0.05$ in comparison to the untreated dendritic cells. Inactivated refers to *V. cholerae* cultures that were treated with a combination of heat and 1.5% formalin. Exposed refers to *V. cholerae* cultures that were solar irradiated. Dark refers to *V. cholerae* cultures that were non-solar irradiated. PBS refers to *V. cholerae* cells that were prepared in PBS.

Table 2 | Surface percentage expression of the maturation makers of the dendritic cells*

DC Treatment	CD 40 Mean ± SE	CD54 Mean ± SE	CD80 Mean ± SE	CD83 Mean ± SE	CD86 Mean ± SE	MHC-I Mean ± SE	MHC-II Mean ± SE
Untreated	27.18 ± 1.90	30.28 ± 0.35	30.58 ± 0.93	39.02 ± 1.37	24.98 ± 2.45	29.72 ± 0.34	30.88 ± 0.64
LPS	62.05 ± 3.22^{a,b,c,d}	79.21 ± 3.19^{a}	27.42 ± 4.16	60.73 ± 0.34^{a}	73.88 ± 1.32^{a}	71.75 ± 3.05^{a}	64.65 ± 3.60^{a}
CTB	41.88 ± 0.73	57.68 ± 0.97^{a}	56.65 ± 3.65^{a}	67.63 ± 1.02^{a}	54.13 ± 5.80^{a}	60.65 ± 3.26^{a}	49.42 ± 4.61^{a}
G4222-In PBS	37.83 ± 1.45	50.33 ± 5.24^{a}	62.35 ± 8.85^{a,b}	36.73 ± 12.08	39.32 ± 5.79	39.20 ± 11.70	44.23 ± 4.61
G4222-Inactivated	4.17 ± 1.12^{a,b}	33.55 ± 8.35^{b}	1.73 ± 1.34 ^{a,b}	69.35 ± 0.95^{a,b}	2.09 ± 1.31^{a,b}	3.76 ± 0.78^{a,b}	39.48 ± 6.95
G4222-Exposed	41.13 ± 1.49	58.42 ± 1.65^{a}	32.80 ± 1.01	47.58 ± 0.63	53.12 ± 2.05^{a}	55.50 ± 3.67	44.58 ± 5.03
G4222-Dark	44.93 ± 4.40^{a}	13.71 ± 3.68^{b}	15.35 ± 2.11	23.57 ± 0.63^{b}	62.45 ± 1.75^{a}	28.20 ± 6.33^{b}	42.92 ± 1.40
BRITS01-In PBS	47.92 ± 5.59^{a}	54.83 ± 4.67^{a}	44.92 ± 4.71	65.93 ± 0.08^{a,c}	46.97 ± 2.93^{a}	48.30 ± 7.63	45.92 ± 1.38
BRITS01-Inactivated	13.41 ± 2.22^{c}	16.46 ± 4.42^{c}	43.87 ± 8.08	42.40 ± 6.60	3.43 ± 0.93^{c}	4.89 ± 1.62^{a,c}	51.30 ± 2.15^{a}
BRITS01-Exposed	40.38 ± 2.01	55.30 ± 2.89^{a}	30.00 ± 1.29	44.43 ± 3.61	53.97 ± 0.96^{a}	46.78 ± 1.69	34.92 ± 2.75
BRITS01-Dark	52.93 ± 3.88^{a}	21.23 ± 3.86^{c}	11.91 ± 2.85	30.40 ± 2.68	64.37 ± 4.62^{a}	37.30 ± 3.50	46.87 ± 2.28
ENV1009-In PBS	43.08 ± 2.78	27.03 ± 9.63^{d}	28.95 ± 3.05	43.63 ± 11.83	39.68 ± 11.43	18.78 ± 1.88^{d}	62.20 ± 2.30^{a,d}
ENV1009-Inactivated	44.55 ± 6.65^{a}	6.32 ± 3.70^{a,d}	40.20 ± 15.60	51.65 ± 4.15	75.22 ± 5.94^{a}	3.73 ± 0.56^{a,d}	59.13 ± 5.03^{a,b}
ENV1009-Exposed	39.87 ± 1.00	58.40 ± 3.53^{a}	31.17 ± 0.17	47.68 ± 1.18	56.30 ± 1.15^{a}	49.50 ± 0.97	33.27 ± 1.10
ENV1009-Dark	53.60 ± 2.82^{a}	6.23 ± 1.69^{a,d}	6.44 ± 0.48^{a,d}	20.54 ± 2.92^{a,d}	58.88 ± 1.66^{a}	38.77 ± 0.56	45.20 ± 1.49

*Percentages of JAWS II cells expressing the surface markers are given from pooled data of three independent experiments. $P < 0.05$ in comparison to; [a] untreated; [b] G4222-Exposed, [c] BRITS01-Exposed and [d] ENV1009-Exposed. Inactivated refers to *V. cholerae* cultures that were treated with a combination of heat and 1.5% formalin. Exposed refers to *V. cholerae* cultures that were solar irradiated. Dark refers to *V. cholerae* cultures that were non-solar irradiated. PBS refers to *V. cholerae* cells that were prepared in PBS.

readings from all the treatment groups. The dendritic cells were incubated for 48 h with LPS, CTB and *V. cholerae* cultures that were solar irradiated, non-solar irradiated, prepared in PBS and inactivated through the use of heat and 1.5% formalin. The untreated (unstimulated) but stained dendritic cells were used to establish the cut off point for what was considered positive.

The use of the unstimulated dendritic cells as the biological comparison control was due to their ability to account for spill over effects on the channels of interest. Furthermore, the unstimulated dendritic cells, just like the isotype controls, could account for nonspecific staining in the channel of interest (O'Gorman & Thomas 1999; Maecker & Trotter 2006). LPS-treated dendritic cells were used as a positive control because LPS is known to induce dendritic cell maturation (Cao *et al.* 2005). The use of LPS as a positive control provided experimental validation, and also guarded against the misinterpretation of negative data (Maecker & Trotter 2006). A one-way ANOVA among subjects was conducted to compare the effect of LPS, CTB and different *V. cholerae* treatments on the expression of the maturation markers on the dendritic cells. There was an increase in the expression of all the maturation markers (CD40, CD54, CD83, CD86, MHC-I and MHC-II) with the exception of CD80 in dendritic cells which were incubated with LPS (Table 2). The increase in these maturation markers was significantly higher (at a $P < 0.05$ level) than that observed in the untreated dendritic cells (Table 2). CTB stimulated dendritic cells also showed an increase in the expression of all the maturation markers. However, only an increase in the expression of CD54, CD80, CD83, CD86, MHC-I and MHC-II was significant at a $P < 0.05$ level in comparison to those observed in the untreated dendritic cells (Table 2).

In contrast to the untreated cells, the dendritic cells incubated with solar irradiated cultures of *V. cholerae* showed an expression profile of the maturation markers in which CD54, CD86 and MHC-I were highly expressed (Table 2 and Figure 2); CD83 and CD40 moderately expressed and MHC-II and CD80 the least expressed (Table 2). This confirms that the level of expression of these maturation markers was higher in dendritic cells incubated with solar irradiated cultures of *V. cholerae* in comparison to the untreated dendritic cells (Table 2).

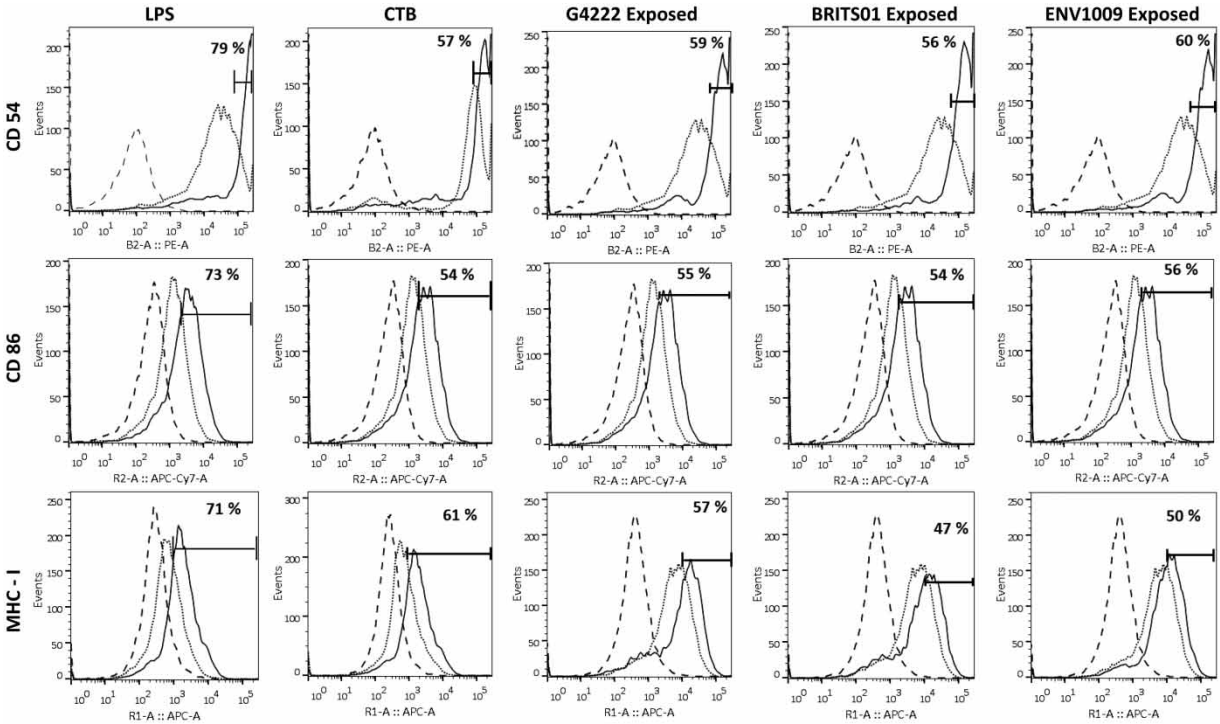

Figure 2 | Stimulation of DC with SODIS treated *V. cholerea* in water results in cell maturation characterised by high expression of CD54, CD86, and MHC class I. The broken line represent unstimulated and unstained dendritic cells, the dotted line represents unstimulated but stained dendritic cell and the solid line represents the stimulated and stained dendritic cells. The flow cytometry histograms are a representative of three independent experiments.

However, only the expression of CD54 and CD86 was significant at a $P < 0.05$ level when compared to that of the untreated dendritic cells (Table 2). The expression of CD80 was minimal and close to that observed in the untreated dendritic cells.

The dendritic cells that were incubated with the solar irradiation control (dark) cultures of *V. cholerae* showed an expression profile where maturation markers CD86 and CD40 were highly expressed; MHC-II moderately expressed and MHC-I the least expressed (Table 2). The expression of CD40 and CD86 in stimulation experiments where non-solar irradiated *V. cholerae* was used was significant at a $P < 0.05$ level when compared to that of the untreated dendritic cells (Table 2). A down regulation in the expression of CD54, CD80 and CD83 was observed in co-incubation experiments where non-solar irradiated *V. cholerae* was used (Table 2). However, only the ENV1009 strain seemed to have induced a significant down regulation of these maturation markers at a $P < 0.05$ level when compared to that of the untreated dendritic cells (Table 2).

Dendritic cells that were co-incubated with viable PBS prepared cultures of *V. cholerae* did not have a distinct pattern in the expression of the maturation markers such as that observed in stimulation experiments involving the solar (exposed) or non-solar irradiated (dark) cultures (Table 2). The maturation marker expression profile in the dendritic cells co-incubated with the PBS prepared cultures of *V. cholerae* was strain specific (Table 2). The toxigenic strains induced an increase in the expression of all the maturation markers with the exception of CD83 in the G4222 strain (Table 2) where a slight decrease in expression was observed. The increase in expression of CD54 and CD86 in dendritic cells that were stimulated with PBS prepared G4222 was significantly higher than that of the unstimulated dendritic cells at a $P < 0.05$ level (Table 2). Dendritic cells that were co-incubated with PBS prepared BRITS01 showed a significant increase in the expression of CD83, CD54, CD86 and CD40 at a $P < 0.05$ level when compared to the untreated dendritic cells (Table 2). The ENV1009 strain of *V. cholerae* induced a significant increase in the

expression of MHC-II. However, there was a down regulation in the expression of CD54, CD80 and MHC-I (Table 2).

The dendritic cells that were co-incubated with heat and chemically inactivated cultures of *V. cholerae* showed an increase in the expression of CD83 and MHC-II (Table 2). However, the expression of the other maturation markers was strain specific (Table 2). The ENV1009 strain induced an increase in the expression of CD40 ($P < 0.05$), CD86 ($P < 0.05$) and CD80, when compared to the unstimulated dendritic cells. The same strain also induced a significant down regulation in the expression of CD54 and MHC-I at a $P < 0.05$ level when compared to the unstimulated dendritic cells. Dendritic cells that were co-incubated with the BRITS01 strain showed an increase in the expression of CD80 and a decrease in the expression of CD40, CD54, CD86 and MHC-I ($P < 0.05$) when compared to the unstimulated dendritic cells. The G4222 strain of *V. cholerae* significantly down regulated the expression of CD40, CD80, CD86 and MHC-II at a $P < 0.05$ level in comparison to the unstimulated cells.

The expression of the maturation markers on the dendritic cells resulting from co-incubation with viable PBS and water (SODIS control) prepared cultures of *V. cholerae* was different (Table 2). Although the pattern of expression of these markers was similar, the levels at which they were expressed differed. There was a down regulation in the expression of CD54 and CD80. This was observed in dendritic cells that were stimulated with water prepared (SODIS control) cultures of the toxigenic strains of *V. cholerae*. Dendritic cells that were co-stimulated with the same toxigenic strains of *V. cholerae* but prepared in PBS showed an increase in the expression of these maturation markers. There was a down regulation in the expression of these markers by the dendritic cells that were stimulated with the ENV1009 strain of *V. cholerae* prepared in water (SODIS control) or PBS (Table 2). The water prepared (non-solar irradiated) strains of *V. cholerae* induced a greater increase in the expression of CD40 and CD86 in dendritic cells when compared to those prepared in PBS. These observations show that the medium in which the microorganisms are prepared may have had an influence on the type and quality of *V. cholerae* antigens.

DISCUSSION

The role played by the host immune system following the consumption of solar irradiated water pathogens has not received any significant attention. This study investigated the potential for solar inactivated *V. cholerae* to induce the maturation of the dendritic cells *in vitro*. Dendritic cells are professional antigen presenting cells with the capacity to bridge the gap between the innate and adaptive immune system. The ability of the dendritic cells to carry out their function depends on their maturity level. Maturation of the dendritic cells enables them to physically interact with T-cells thus inducing T-cell activation. Immature dendritic cells are unable to efficiently and effectively present antigens due to low levels in the expresion of phenotypic maturation markers such as MHC-I, MHC-II and co-stimulatory molecules.

In this study, a hypervirulent (G4222), a hypovirulent (BRITS01) and an environmental (ENV1009) strain of of *V. cholerae* were used. When these strains where solar irradiated, a total loss in their culturability was observed (Figure 1). The loss in culturability was attributed to the ability for SUVR to form photosensitisers such as the superoxide and hydroxyl radicals as well as hydrogen peroxide and singlet oxygen. Studies have shown that the interaction between the photosensitisers and actively growing microbial cells results in irreversible damage to the catalase system (Alonso-Sáez *et al.* 2006), inactivation of the electron transport systems (Bosshard *et al.* 2010a), single strand breaks in DNA (Bosshard *et al.* 2010b) and induced damage to the cell membrane (Berney *et al.* 2006; Ssemakalu 2011). The period within which the microbial inactivation occurred was considerably shorter than that observed during winter or spring at the point of exposure (Ssemakalu *et al.* 2012). Quick inactivation observed during summer was a result of the synergy between the increased amount of solar ultraviolet radiation and temperature at the point of exposure (Figure 1).

When the dendritic cells were co-incubated with the solar irradiated cultures of *V. cholerae*, an increase in the expression of the maturation markers was observed (Table 2 and Figure 2). Solar irradiated cultures of *V. cholerae* provided an appropriate signal required for the upregulation of CD54 (Figure 2) in dendritic cells suggesting that these cells are capable of interacting with the LFA-1 molecule on T-cells. This interaction acts as a co-activation

signal and is thought to promote the induction of type 1 immune cells independent of a signal from IL-12 (Lavelle *et al.* 2003). On the contrary, there was a downregulation in the expression of CD54 by the dendritic cells stimulated with *V. cholerae* cultures that had been non-solar irradiated as well as those that had been inactivated by a combination of heat and chemical means. This means that these treatments resulted in a signal that could have blocked the CD54 induction pathway through the production of proteosome inhibitors. Proteosome inhibitors have been reported to inhibit CD54 expression in endothelial cells through their action on TNF-α (Roebuck & Finnegan 1999). CD54 is associated with a variety of inflamatory conditions such as asthma, inflamatory bowel syndrome, acute respiratory distress syndrome and autoimmune disease (Roebuck & Finnegan 1999).

Besides CD54 there was an upregulation in the expression of CD86 (Figure 2) by the dendritic cells stimulated with solar irradiated strains of *V. cholerae*. The same was observed in dendritic cells co-incubated with non-solar irradiated cultures of *V. cholerae*. CD86 plays a critical role in strengthing the connection between the antigen presenting cell and the T-cell through its interaction with CD28 or the cytotoxic T lymphocyte associated protein-4 (CTLA-4) on the T-cell. The interaction between CD86 and CD28 is required for the induction of an adaptive immune response whereas that between CD86 and CTLA-4 has been found to inhibit T-cell receptor and CD28 signalling (West *et al.* 2011). Upregulation of CD86 in the dendritic cells treated with solar irradiated *V. cholerae* could result in the inhibition or activation of an immune response.

There was increased expression of both MHC-I and II molecules in the dendritic cells stimulated with solar irradiated cultures of *V. cholerae*. However, the expression of MHC-I was greater than that of MHC-II. This finding is unusual because the MHC-I pathway is predominantly used by intracellular pathogens and yet *V. cholerae* is not an intracellular pathogen. However, it is possible that the *V. cholerae* antigen could have been presented on MHC-I through cross presentation. Cross presentation offers a means by which tumor antigens and microbes that do not necessarily infect dendritic cells can be recognised (Trombetta & Mellman 2005). This result seems to suggest that dendritic cells stimulated with solar irradiated *V. cholerae* are capable of engaging and activating CD8+ T cells.

The expression of CD83 and CD40 on the dendritic cells co-incubated with solar irradiated cultures of *V. cholerae* was moderate in comparison to those that were unstimulated or co-stimulated with LPS (Table 2). CD83 has been considered as the hallmark of dendritic cell maturation (West *et al.* 2011). This maturation marker has been reported to enrich and amplify antigen specific CD8+ T cells (Jonuleit *et al.* 2001; Cao *et al.* 2005). The expression of CD83 was down regulated in dendritic cells stimulated with non-solar irradiated cultures of *V. cholerae*. Down regulation of CD83 on dendritic cells reduces their capacity to prime antigen specific CD8 + T lymphocytes (Aerts-Toegaert *et al.* 2007). Solar irradiated cultures of *V. cholerae* also induced a moderate increase in the expression of CD40 in comparision to the unstimulated control and LPS in dendritic cells. However, the level of induction of CD40 on the dendritic cells was slightly lower than that induced by the non-solar irradiated cultures of *V. cholerae* (Table 2). The interaction between the expressed CD40 with its ligand has been reported to increase the efficiency with which dendritic cells carry out their function (O'Sullivan & Thomas 2003; Ma & Clark 2009). Dendritic cell CD40 ligation upregulates the expression of MHC-II, CD80, and CD86. It also up regulates the production of cytokines such as IL-12p40 and IL-6, thereby inducing a T helper type 1 immune response. Failure to express CD40 and/or CD54 after stimulation results in regulatory dendritic cells. The generation of regulatory T cells, although undesired in vaccines, may be beneficial because it is needed in the prevention of autoimmune diseases and inflammatory related conditions.

The down regulation of CD54, CD80 and CD83 in dendritic cells stimulated with non-solar irradiated cultures of *V. cholerae* could result in the lack of an immune reaction. The production of compounds that down regulate the expression of key dendritic cell maturation markers could be a strategy used by pathogens to evade the immune system. Evidence showing the ability of infectious pathogens to evade a type 1 immune response is available. Studies showed that pathogens are able to evade an immune response by inducing the production of regulatory T-cells (Jonuleit *et al.* 2001; Lavelle *et al.* 2003).

The levels in the expression of the different maturation markers on the dendritic cells seemed to have been influenced by the medium in which the cultures of *V. cholerae* were prepared as well as the strain. *Vibrio cholerae* cultures prepared in PBS induced an up-regulation in the expression of CD54,

CD80 and CD83 maturation markers. However, expression of the same markers was downregulated when dendritic cells were incubated with viable but non-solar irradiated cultures prepared in water. PBS could have provided an isotonic environment and hence was non-detrimental to the physiological state of the microrganisms. The water environment, due to lack of proper buffering, could have induced the expression of microbial products that could have antagonised the expression of some of the maturation markers.

In conclusion, our observations suggest that the phenotype of the dendritic cells generated following co-incubation with solar irradiated *V. cholerae* is capable of eliciting a T-cell type 1 immune response. However, mixed leukocyte activation assays are also required to definitively show the exact phenotype of T-cells activated by solar disinfected *V. cholerae* in water. In addition, further studies looking at the expression profile of the toll-like receptors as well as cytokine and chemokine secretion by the dendritic cells are required. These studies could help explain the pathways used to induce the maturation of the dendritic cells. This study presents information that is of relevance for understanding the possible immunological consquences of consuming SODIS treated water.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

CCS concieved the idea. EU-J, KSCMM and MP made intellectual contributions. CSS and KSCMM designed the study and carried out the experiments. CCS, EU-J, KSCMM and MP analysed the data. CCS wrote the manuscript. EU-J and MP critically reviewed the manuscript with subsequent intellectual input. All authors read and approved the final manuscript.

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