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Electrochemotherapy impacts the viability, functionality and phenotype of murine dendritic cells in voltage-dependent manner

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ABSTRACT

Dendritic cells (DCs) are central to the initiation of anti-tumour immunity, yet the direct effects of electrochemotherapy (ECT) on DC biology remain poorly defined. We investigated how varying electric field strengths (800, 1000, and 1300 V/cm) modulate murine DC viability, phenotype, Toll-like receptor (TLR) expression and functional responses *in vitro*. ECT induced predominantly necrotic cell death in a voltage-dependent manner, with higher voltages markedly reducing viability. Despite this decline, surviving DCs exposed to 1000 V/cm and 1300 V/cm upregulated MHC II and CD80, indicating activation of the remaining population. ECT also altered TLR expression, significantly increasing TLR6 and TLR9 while reducing TLR4, TLR7 and TLR8 levels. Functionally, ECT impaired DC cytokine responses to TLR4 and TLR7/8 stimulation but preserved or enhanced responsiveness to the TLR9 agonist CpG, suggesting selective disruption of downstream TLR pathways. Together, these findings demonstrate that ECT differentially shapes DC survival and function depending on electric field intensity, with low-voltage ECT better preserving DC competence and higher voltages inducing activation but impairing responsiveness. These data highlight opportunities to optimise ECT protocols and rationally combine ECT with immune adjuvants to enhance therapeutic immunogenicity.

1. Introduction

Electrochemotherapy (ECT) is a clinically established locoregional cancer treatment that combines short, high-voltage electric pulses with chemotherapeutic agents such as bleomycin or cisplatin to enhance intracellular drug uptake [1]. Over three decades of clinical use, ECT has demonstrated increased response rates in cutaneous and subcutaneous malignancies, including melanoma, breast cancer metastases and head-and-neck tumours [2]. Traditionally viewed as a tumour-ablative technique, ECT achieves rapid tumour cell death through membrane permeabilisation and drug potentiation, alongside secondary vascular disruption that in turn contributes to tumour reduction [3].

Although initially developed for efficient drug delivery and tumour ablation, ECT is now increasingly recognised for its capacity to provoke systemic immune effects. Increasingly, ECT is recognised as a potent inducer of immunogenic cell death (ICD), a regulated form of cell death characterized by the release of damage-associated molecular patterns (DAMPs) such as ATP, HMGB1 and surface-exposed calreticulin [4]. These signals can recruit and activate innate immune cells, promote dendritic cell (DC) maturation, and facilitate the priming of tumour-specific cytotoxic T lymphocytes. In this way, ECT has the capacity to transform the treated lesion into an *in situ* tumour vaccine.

Both preclinical and human studies have shown that ECT elicits changes in the immune cell composition in both the treated areas as well as at distant sites [1,5]. Preclinical murine studies have shown that bleomycin-based ECT transiently increases infiltration of DCs, T cells, NK cells and macrophages at both treated and distant sites [6,7]. In melanoma patients, bleomycin ECT enhances infiltration of cytotoxic $CD8^+$ T cells and plasmacytoid DCs whilst reducing immunosuppressive $CD4^+$ $FOXP3^+$ regulatory T cells and Langerhans cells (skin-specific subtype of DCs) [8,9]. Additionally, Roux et al. showed that electro-immunotherapy, consisting of ECT together with the TLR9 agonist

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CpGDNA, can increase local and systemic immune responses in murine melanoma models and a murine fibrosarcoma model, as well as induce a specific immune memory that is T cell dependent [10]. In terms of direct impacts on immune cells, calcium electroporation (CaEP) has been shown to increase the ability of melanoma-conditioned bone marrow-derived macrophages to stimulate cytotoxic $CD8^+$ T cells [11]. Most recently, Valentinuzzi et al. undertook a comprehensive *in vivo* study on the effects of ECT with three different chemotherapeutic agents on two tumour types (B16F10 melanoma and 4 T1 breast) [12]. These authors demonstrated that ECT in the 4 T1 tumour model exhibited a more pronounced immune response relative to the B16 melanoma model, with an abscopal effect only observed in the 4 T1 model. Nevertheless, the direct effects of ECT on immune cells have, to date, been incompletely characterized.

In order for ECT to function effectively, not just as a tumour ablative technique, but also as an immune response-enhancing modality, tumour antigens released during ICD must be efficiently captured and processed by professional antigen-presenting cells. Dendritic cells (DCs), as central orchestrators of anti-tumour immunity governing antigen acquisition, T-cell priming and activation of additional immune effector cells, are uniquely equipped for this role. In the context of cancer, DCs are indispensable for mounting effective anti-tumour immunity. When appropriately activated and loaded with tumour-derived antigens, DCs migrate to draining lymph nodes, cross-present tumour-associated antigens to cytotoxic lymphocytes, and thereby initiate antigen-specific adaptive responses [13]. Despite this link between ECT-mediated tumour cell death and tumour adjacent DC-mediated antigen uptake and presentation, the direct effects of ECT on DCs remain insufficiently understood.

We previously demonstrated that murine DC viability declines sharply after EP at field strengths above 800 V/cm *in vitro* [14], suggesting that standard ESOPE ECT protocols (~1000 V/cm) may compromise viability of local antigen-presenting cells. In this study, we systematically investigated the cellular and molecular responses of murine DCs to ECT at varying electric field strengthsThe electric field strengths (800, 1000 and 1300 V/cm) were selected to encompass the range of intratumoural electric fields encountered during clinical electrochemotherapy, with 1000 V/cm corresponding to the standard ESOPE protocol [15].The 800 V/cm condition was included to model a lower-bound electric field strength that may occur *in vivo* due to tissue heterogeneity, field attenuation, and distance from electrodes, and to explore whether reduced electric fields better preserve immune cell viability and function as based on our previous findings [14]. The 1300 V/cm condition represents the upper range of electric field intensities that can occur locally within tumours during clinical ECT, accounting for non-uniform field distributions and electrode-dependent field amplification [16]We examined the mode of cell death, phenotypic and Toll-like receptor (TLR) expression changes, and functional capacity through cytokine secretion profiles. Our findings aim to clarify how ECT influences immune cell biology and guide optimization of ECT protocols to enhance anti-tumour immunity.

2. Materials & methods

2.1. Murine bone marrow-derived dendritic cell isolation

Following ethical approval from UCC's Animal Experimentation Ethics Committee, application ID 20/2004, bone marrow-derived cells were obtained from the back legs of healthy 8–20-week-old female C57BL6J mice (Envigo, Bicester, UK) by passing ice cold RPMI through the bones to obtain the bone marrow, which was then passed through a 0.45 μM cell strainer (BD, Falcon, Franklin Lakes, USA) to obtain the cells. Red cells were lysed with red cell lysis buffer for 3 min on ice (Biolegend, San Diego, USA). Bone marrow cells were diluted at 1×10^6 cells/mL in RPMI with 10% heat inactivated FBS, 50IU/mL penicillin and 50μg/mL streptomycin supplemented with 10 ng/mL GM-CSF (Peprotech, London, UK) and 10 ng/mL IL-4 (Peprotech). Cells were cultured at 4 mL/well in 6 well plates for a total of 6 days. Cells were split in half on day 2 and day 4. Non-adherent cells on day 6 were considered to be bone marrow-derived Dendritic cells (DCs).

2.2. In vitro EP and ECT parameters

1×10^6 cells were suspended in 400μL of electroporation buffer (EPB: 10 mM HEPES, 250 mM Sucrose and 1 mM $MgCl_2$ in ddH_20) either with 11 μM cisplatin (ECT) or without cisplatin (EP). Cell suspensions were aliquoted into 4 mm sterile EP cuvettes (VWR). Cuvettes were exposed to eight electric pulses at 800 V/cm, 1000 V/cm and 1300 V/cm electric field intensities respectively, with pulse duration of 100 μs and 1 Hz frequency using a BTX ECM 2001 square wave electroporator (BTX, San Diego, USA). After the electroporation, cells were rested for 20–30 min at 37 °C and 5% CO_2 prior to subsequent analyses.

2.3. Flow cytometry

100,000 to 200,000 cells were diluted in 100 μL FACS buffer (PBS supplemented with 5% heat-inactivated FBS and 2 mM EDTA), and non-specific binding of the cell surface receptors CD16/CD32 was blocked using TruStain FcX™ Antibody (Biolegend). The cells were then stained for 30 min at 8 °C in the dark with Fixable live-death marker Near-infrared (Thermofisher Scientific), AF488 Anti-CD11c (N418; Biolegend), PerCP-Cy5.5 Anti-CD80 (16-10A1; Biolegend) and APC Anti-MHC11 (M5/114.15.2; Biolegend). Following washing, expression of MHC11, CD11c and CD80 was detected by Flow cytometry at both 4 h and 24 h post treatment, with at least 10,000 events collected per sample. Flow cytometry was conducted using an LSRII machine with a 5-laser (355 /405 /488 /561 /640 nm) configuration (BD Biosciences) and the results analysed using FlowJo V10 (BD Biosciences). Cells were first gated on forward scatter (FSC) *vs.* side scatter (SSC) to exclude debris and then gated on FSC-area *vs.* FSC-high to exclude doublets. Following this, dead cells were excluded by excluding cells positive for Fixable live-death marker Near-infrared. CD11c and MHC11 were subsequently analysed using bivariate plots and within the $CD11c^+MHC11^+$ positive population the CD80 expression was analysed (Supplemental Fig. 1).

2.4. Viability assay

The viability of electroporated cells was tested 24 h post treatment. Following plating for 24 h, cells were transferred into FACS tubes, PI added, and PI incorporation analysed by Flow cytometry.

2.5. Cell death analysis

Cell death was assessed 4 h post ECT treatment. Cells were washed once in FACS buffer, stained with APC Annexin V and propidium iodine (Biolegend) for 15 min according to the manufacturer's protocol, and analysed by Flow cytometry.

2.6. qRT-PCR

RNA was isolated 4 h post-treatment utilizing the GenElute total mRNA kit (Sigma Aldrich) as per manufacturer's guidelines. cDNA was synthesized (Bioline cDNA synthesis kit (Bioline, London, UK), and subsequently amplified on the LightCycler® 480 (Roche, Basel, Switzerland). All results were analysed using the ΔΔct method and gene expression normalized to the corresponding β-actin gene results. Fold-change values over the untreated condition are represented and at least 3 separate biological replicas were analysed.

2.7. Enzyme-Linked Immunosorbent Assays (ELISA)

DCs were rested for 30 min post-treatment before being stimulated

with either 1 μg/mL LPS (Invivogen, San Diego, USA), 1 μM CpG (ODN2395; Invivogen), 10 μg/mL polyI:C (Invivogen) or 1 μg/mL R848 (Invivogen) for 24 h. After 24 h, cell supernatants were collected and either stored at −20 °C or used directly. The concentration of murine IFNγ and TNFα was determined using ELISA MAX Deluxe Kits, according to manufacturer's manual (Biolegend).

2.8. Statistical analysis

Results were statistically evaluated using one-way ANOVA followed by Dunnett's post-hoc test using GraphPad Prism version 9.0 (GraphPad Software, San Diego, USA). Outliers were detected using Grubbs' test. Statistical significance was defined as $p \leq 0.05$.

3. Results

3.1. EP and ECT leads to necrotic cell death in DCs

To investigate the impact ECT has on DCs, we first established the impact of the varying electric field strengths on DC viability using electroporation (EP) at 800 V/cm, 1000 V/cm and 1300 V/cm (Fig. 1a). As we have shown previously [14], the viability of DCs 24 h post-treatment decreased with increasing electric field strengths. Given this reduction in cell viability, we next determined the type of cell death that the DCs undergo following EP utilizing the Annexin V- propidium iodine (PI) apoptosis assay [17]. Cells which stain positive only for Annexin V are considered apoptotic cells, while cells staining double-positive for Annexin V and PI are considered late apoptotic cells, with PI-only positive cells considered to be necrotic [18]. This analysis revealed that DCs undergo necrotic cell death rather than apoptotic cell death 24 h post EP (Fig. 1b). Moreover, with increasing electric field strengths, an increase in the % of necrotic cells was noted, mirroring the reduction in viability. The impact of ECT with 11 μM cisplatin at the different electric field strengths on the viability of DCs was next assessed (Fig. 1c). Similar to the effect of EP on the viability of DCs, all ECT parameters significantly ($p < 0.001$) decreased the viability of the DCs compared to untreated, with the decrease being more pronounced with increasing electric field strengths (Fig. 1c). As per the findings observed regarding EP alone, necrosis was identified to be the mode of cell death (Fig. 1d). Cisplatin alone did not significantly increase the proportion of cells dying *via* necrosis compared to untreated, indicating that the loss in viability is caused mainly by EP, and that the addition of the drug has minimal additional effects.

3.2. ECT at 1000 and 1300 V/cm induce maturation and activation of surviving DCs

To further examine the effect of different electric field strengths on dendritic cells, we next investigated whether the differing voltages, in combination with cisplatin, would alter any of the key cell surface

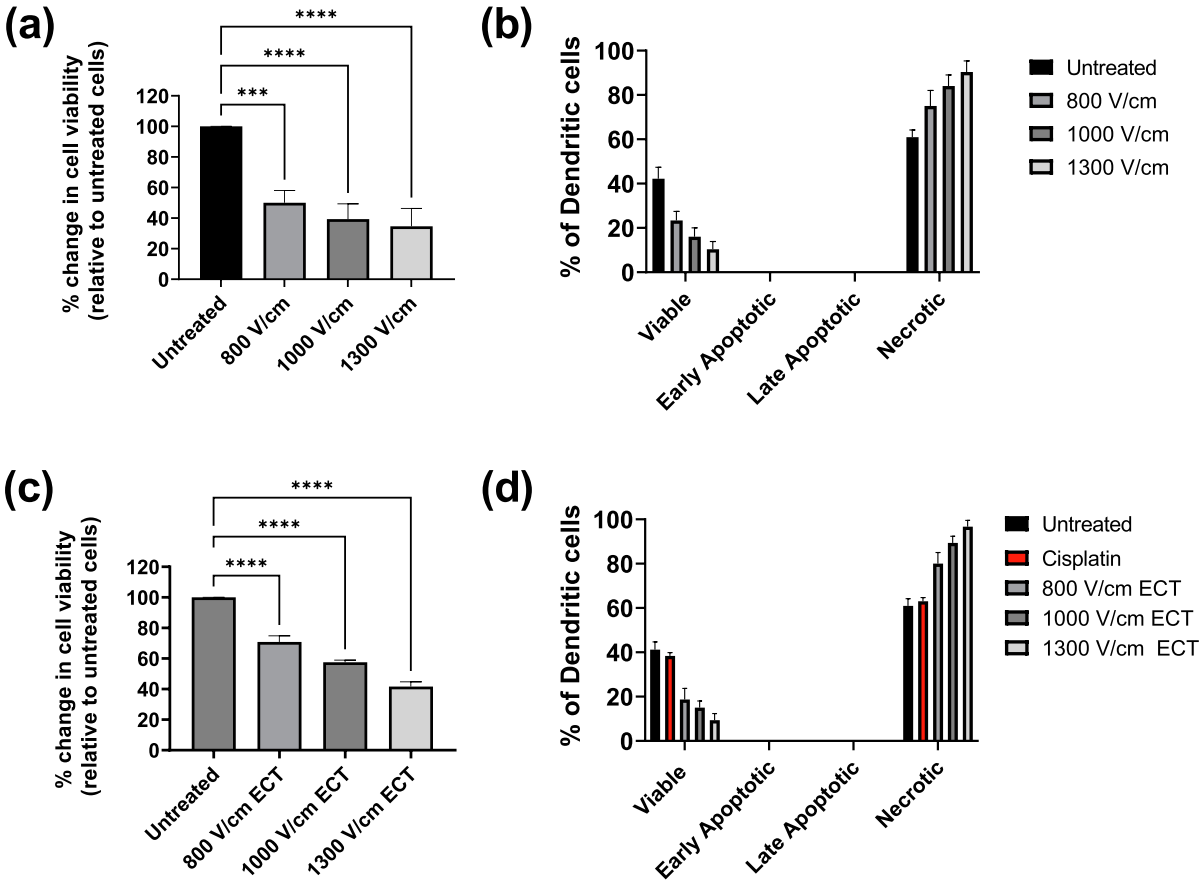


Fig. 1. Electroporation (EP) and electrochemotherapy (ECT) reduce the viability of DCs in a necrotic cell death-dependant manner. DCs were electroporated with/without 11 μM cisplatin at increasing electric field strength, and cell viability measured 24 h post-treatment *via* Flow cytometry. Viability was normalized relative to untreated cells (a, c). Cell death was assessed 4 h post-treatment by AnnexinV and PI staining *via* Flow cytometry. Quantitative plots of flow cytometry data comparing the rate of apoptosis and necrosis of DCs due to EP and ECT (b, d). Data represents the mean ± SD from at least 3 separate experiments. ***$p < 0.001$; ****$p < 0.0001$.

expression markers of dendritic cells. The viability of the DCs post ECT treatment was first determined, with the majority of cells at 4 h being viable (Fig. 2a), but a significant reduction in viability was noted at 24 h (Fig. 2b). The expression of CD11c, MHC II and CD80 was initially assessed 4 h post-treatment in the viable population *via* flow cytometry. 4 h post-treatment, the percentage of MHCII$^+$ CD11c$^+$ DCs and the percentage of activated CD80$^+$ DCs within the MHCII$^+$ CD11c$^+$ subpopulation was seen to be significantly increased by cisplatin in combination with 1300 V/cm ($p \leq 0.01$) (Fig. 2a). 24 hs post-treatment, this effect was found to be enhanced, with the percentage of MHCII$^+$ CD11c$^+$ DCs further increased following ECT at both 1000 V/cm ($p \leq 0.05$) and 1300 V/cm ($p \leq 0.01$), and the percentage of activated CD80$^+$ DCs

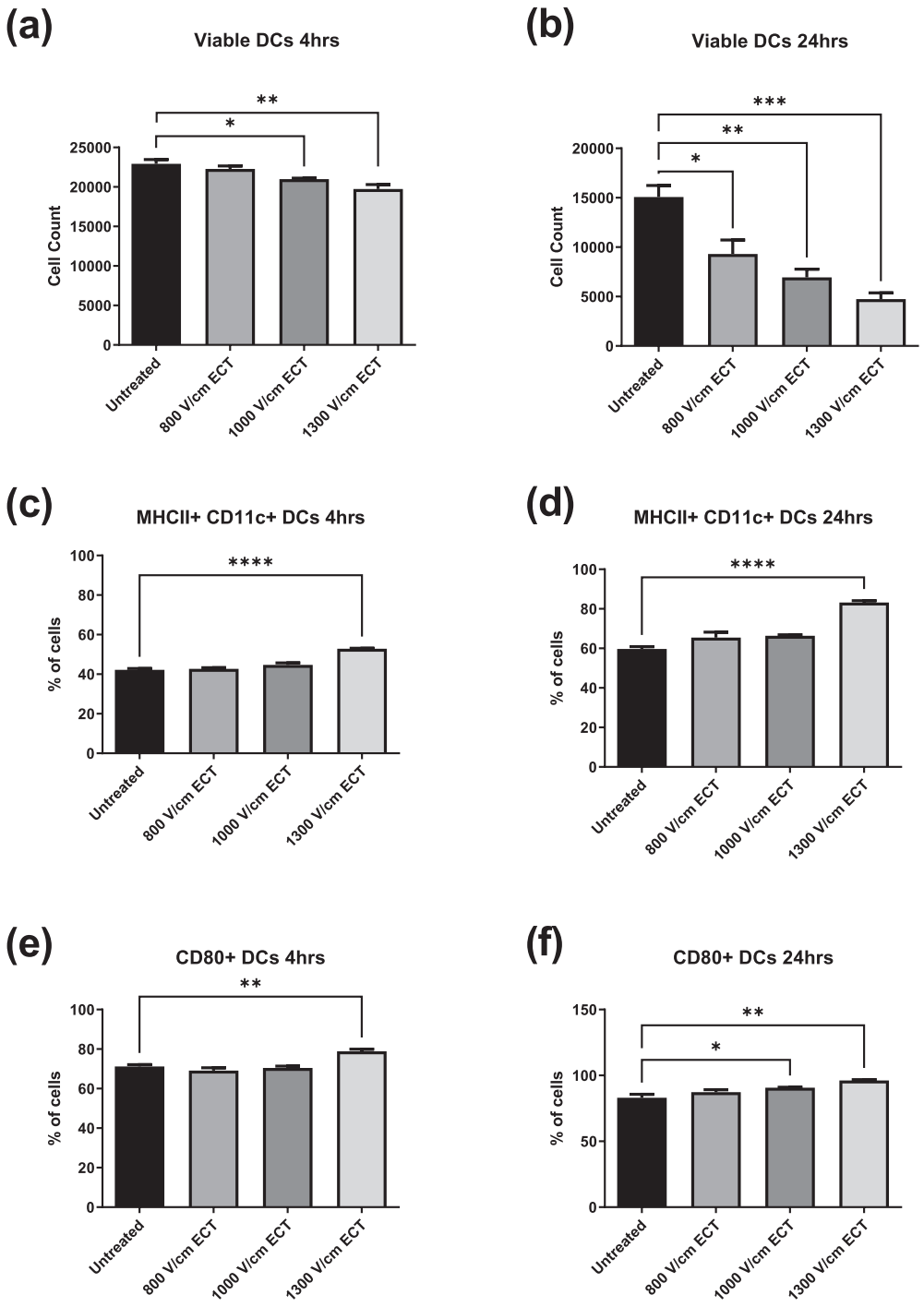


Fig. 2. ECT at 1000 and 1300 V/cm upregulates expression of DC maturation and activation markers. DCs were treated with cisplatin at increasing electric field strengths and the number of viable cells present 4 h (a) and 24 h (b) determined by Flow cytometry. The expression of MHC II, CD11c and CD80 was also determined 4 h (c,e) and 24 h (d,f) post-treatment by flow cytometry. Expression of MHC II$^+$ CD11c$^+$ cells was first determined within the viable population (c,d). CD80 expression was then analysed within the MHC II$^+$ CD11c$^+$ subpopulation (e,f). Data represents the mean ± SD from at least 3 separate experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p \leq 0.0001$.

within the MHCII^{+}CD11c^{+} subpopulation also slightly, but significantly, increased at these voltages (Fig. 2b). In contrast, ECT at 800 V/cm does not induce a significant change in the maturation or expression of activation markers at either 4- or 24-h post-treatment. Thus, whilst ECT decreases the viability of DCs post-treatment in a voltage-dependent manner, ECT also seems to activate the surviving cells at electric field strengths surpassing 1000 V/cm.

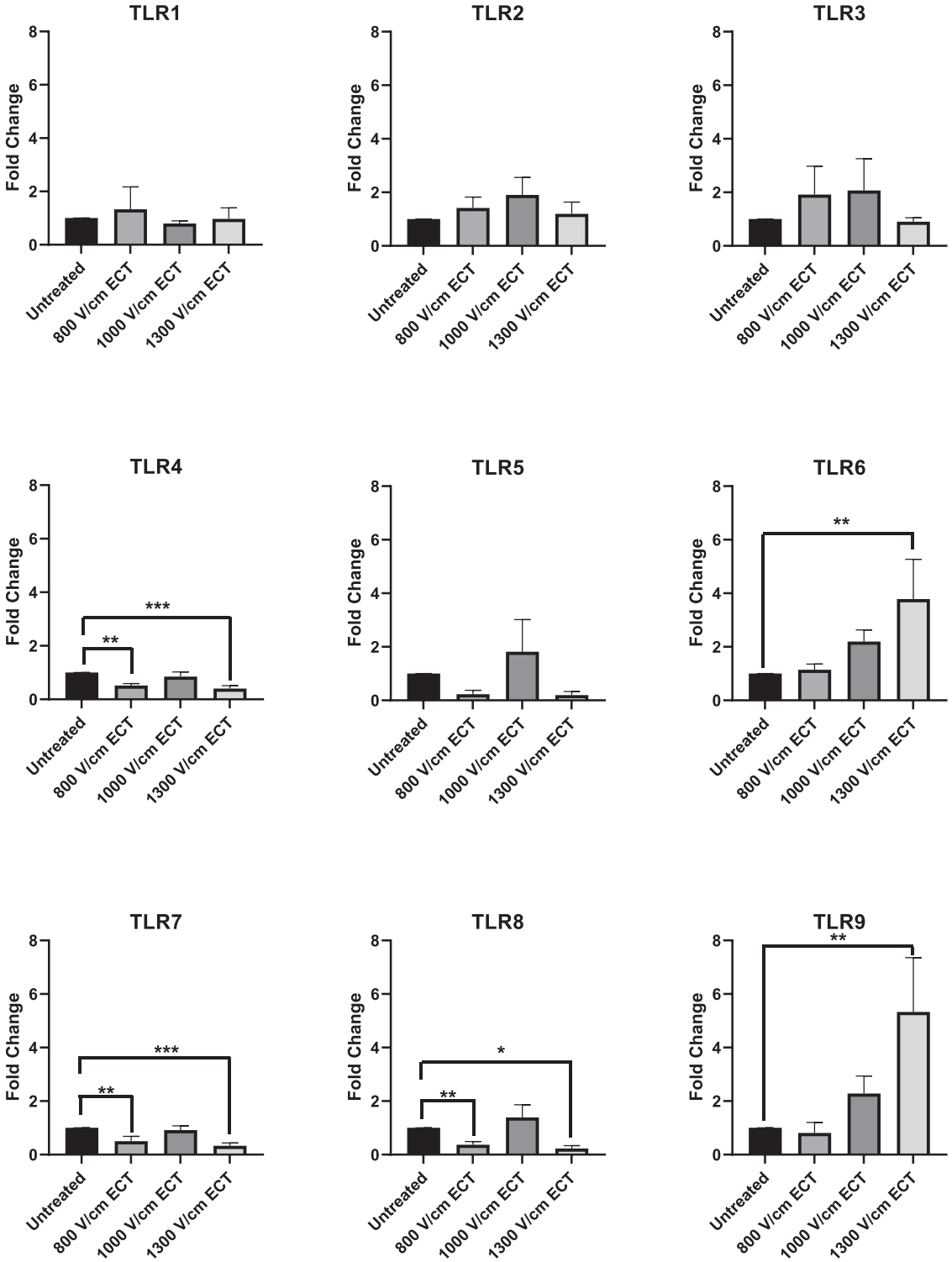


Fig. 3. TLR gene expression by DCs is modified by ECT. DCs were treated with ECT at increasing electric field strength. TLR expression was determined by qRT-PCR 4 h post-treatment. Data represents the mean ± SD for 3 independent experiments. *p < 0.05; **p < 0.01; ***p < 0.001.

3.3. ECT modifies DC expression of TLRs and response to TLR agonists in a voltage dependent manner

Toll-like receptors on DCs play a crucial role in sensing DAMPs released by stressed or dying tumour cells, enabling DCs to mature and effectively capture, process, and present tumour antigens to initiate antitumour immunity. Therefore, in addition to assessing expression of typical DC maturation markers post ECT (Fig. 2), the effect of ECT at different voltages on the mRNA expression of TLRs 1–9 in DCs was examined. Overall, the baseline expression of all 9 TLRs was high as expected for DCs. ECT showed a significant voltage dependant increase in gene expression for TLR6 and TLR9 (Fig. 3), whilst conversely a significant voltage dependant decrease in mRNA expression of TLRs 4, 7 and 8 was observed. ECT did not significantly impact the expression levels of TLR1, 2, 3 or 5.

The functional response of DCs post ECT, in terms of production of key DC-associated cytokines IFNγ and TNFα, was subsequently assessed by ELISA. Whilst DCs were found to basally secrete significantly less IFN-γ following ECT (Fig. 4a) TNFα production post ECT was significantly increased, with cisplatin alone having no impact on the amount of cytokines secreted (Fig. 4a, b). Given the ability of ECT to modify DC expression of certain TLRs, the response of DCs, post ECT treatment, to TLR agonist stimulation was next investigated. DCs were initially stimulated with agonists of TLR 4 and TLRs 7/8, as expression of these three had been found to be reduced post ECT (Fig. 3). Stimulation of DCs with the TLR4 agonist LPS alone increased protein production of both IFNγ and TNFα as expected (Fig. 4c,d). All three voltages reduced the ability of DCs to produce IFNγ in response to LPS to below untreated levels (Fig. 4c,). A more gradual voltage-dependant reduction of TNFα secretion in response to LPS was observed (Fig. 4d), with cells retaining the ability to produce high levels of TNFα at 800 V/cm ECT. A similar pattern was observed following stimulation of ECT-treated DCs with the TLR7/8 agonist R848 (Fig. 4e,f).

The response of DCs to the TLR9 agonist CpG DNA was also examined as, in contrast to the decreased expression of TLRs 4,7 and 8 post ECT treatment, ECT increased mRNA expression of TLR9 on DCs (Fig. 3). Stimulation with CpG DNA alone increased secretion of IFNγ and TNFα (Fig. 4g,h)., However, stimulation of ECT-treated DCs with CpG DNA further increased IFNγ and TNFα secretion, with the induction of IFNγ sustained and slightly enhanced following ECT treatment (Fig. 4g,h). Secretion of TNFα was markedly enhanced at ECT treatment using 800 V/cm, although this level of production was not sustained at the higher voltages (Fig. 4h). These cytokine secretion profiles represent functional readouts of dendritic cell responsiveness following ECT. Taken together, these findings demonstrate that the functional response of DCs to TLR ligand stimulation is both voltage and ligand specific.

4. Discussion

This study provides a comprehensive analysis of how electrochemotherapy (ECT) impacts dendritic cells (DCs) in a voltage-dependent manner, integrating viability, phenotype, gene expression, and functional responses. We show that ECT induces necrotic cell death across all tested voltages, with viability sharply reduced at 1000–1300 V/cm compared to 800 V/cm. Despite this loss, surviving DCs at higher voltages exhibit increased maturation markers (MHCII, CD11c, CD80). In contrast, ECT at 800 V/cm preserved viability but did not enhance maturation, indicating a potential trade-off between cell survival and activation. Selective modulation of TLR expression was identified; TLR6 and TLR9 were upregulated, whereas TLR4, TLR7, and TLR8 were downregulated. Functionally, DCs retained partial responsiveness as stimulation with the TLR9 agonist CpGDNA stimulation sustained IFN-γ and TNF-α secretion post-ECT, whereas responses to LPS (TLR4) and R848 (TLR7/8) were markedly impaired. Basal IFN-γ secretion declined with increasing voltage, while TNF-α secretion was maintained or enhanced at 800 V/cm. Collectively, these findings indicate that ECT exerts dual effects: compromising DC survival while selectively priming innate sensing pathways and cytokine output.

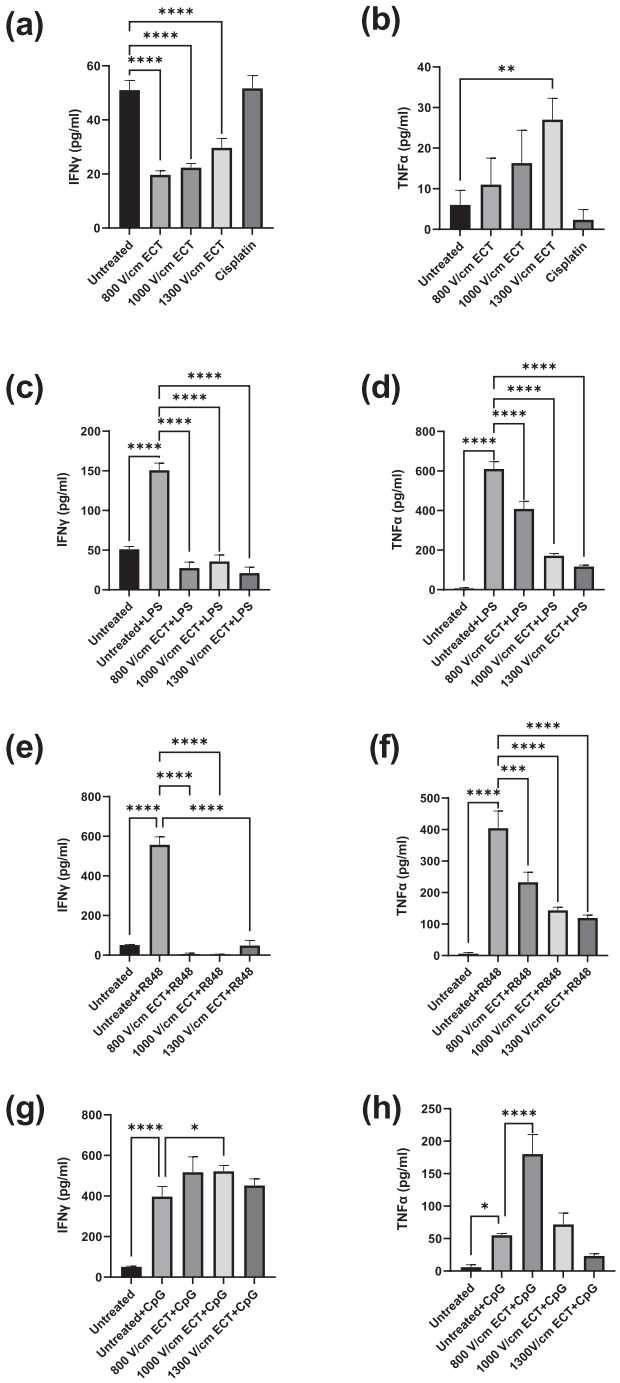


Fig. 4. ECT modifies the secretion of IFN-γ and TNF-α by DCs in response to TLR agonists in an electric field strength and stimulation-dependent manner. DCs were untreated or treated with cisplatin at increasing electric field strength (a, b). DCs were stimulated with/without LPS (TLR4 agonist) (c, d), R848 (TLR7/8 agonist) (e, f) or CpG (TLR9 agonist) (g, h). After24 h, the cell supernatant was collected and secretion of IFNγ and TNFα determined by ELISA (a-h). Data represents the mean ± SD from at least 3 separate experiments. *p < 0.05; ****p ≤ 0.0001.

Cell death analysis *via* flow cytometry employing Annexin V as a marker for apoptotic *versus* non-apoptotic cell death demonstrated that, across all voltages, EP and ECT induced predominantly non-apoptotic cell death as the observed PI-positive/Annexin V–negative phenotype

is consistent with a loss of membrane integrity, which is characteristic of necrotic cell death following electroporation (Fig. 1b, d). While necrosis is classically considered an unregulated, pro-inflammatory death pathway, regulated necrosis-like forms such as necroptosis may also occur [19]. These distinctions are immunologically relevant: different necrotic modalities release distinct DAMPs capable of shaping DC activation and the tumour immune microenvironment [13]. Further studies are required to clarify which specific death pathways are triggered in DCs by EP and ECT, especially as regulated necrosis can enhance anti-tumour immunity [20,21]. These findings align with prior evidence that ECT can trigger immunogenic cell death in tumours [1], potentially converting treated lesions into *in situ* vaccines. However, our data highlight that the direct impact on DCs is complex: while some activation occurs, viability is compromised at clinically relevant voltages.

DCs need to undergo maturation and activation in order to become functionally active, and subsequently able to interact with other innate and adaptive immune cells to enhance the immune response [13]. Thus, it was next investigated whether EP and/or ECT impact the maturation and activation of DCs. Despite pronounced reductions in viability at 1000 V/cm and 1300 V/cm consistent with previously published data [14], surviving DCs were found to upregulate MHC II and CD80 (Fig. 2), canonical markers of maturation. In contrast, ECT at 800 V/cm preserved viability but did not increase activation marker expression. These findings indicate that electric field strength acts as a threshold determinant of DC stress and maturation. Importantly, while low-voltage ECT may not directly induce DC maturation, *in vivo* ECT produces abundant tumour-derived DAMPs capable of activating DCs independently of direct electrical stimulation [20]. Thus, it might not be physiologically significant *in vivo* that ECT at 800 V/cm by itself does not induce DC maturation, as a higher percentage of viable cells following treatment may ultimately result in an increase in the number of activated DC present at the tumour site.

ECT also generated selective and voltage-dependent changes in TLR expression. Notably, alterations in TLR expression were observed even at the lowest electric field strength tested, with reduced expression of TLR4, TLR7 and TLR8 evident at 800 V/cm compared to untreated controls (Fig. 3), indicating that relatively modest electric fields are sufficient to perturb innate immune receptor expression. Upregulation of TLR6 and TLR9, alongside decreased expression of TLR4, TLR7 and TLR8, suggests that electroporation triggers targeted intracellular stress responses rather than generalised innate receptor activation. This is in lines with prior observations showing altered endosomal TLR expression in ECT-treated lesions [10]. The strong induction of TLR9 is particularly notable given the established capacity of unmethylated CpG motifs to drive DC activation [22] and the robust abscopal responses observed when ECT was combined with CpG DNA *in vivo* [10]. The observed upregulation of TLR3 and TLR6 suggests that TLR agonists, other than CpG, in combination with ECT, will improve therapy outcome, although further studies are required to assess this potential.

In addition to examining the above phenotypic changes in DCs post ECT treatment, the functional capacity of the cells was also examined in terms of their ability to respond to TLR agonist stimulation (Fig. 4). Functionally, ECT impaired DC cytokine responses to TLR4 and TLR7/8 stimulation, while responses to CpG were preserved or enhanced, consistent with increased TLR9 expression. This mismatch between receptor expression and cytokine output suggests that ECT may modulate downstream signalling components such as MyD88 or TRIF [23]. Whether this functional impairment arises from metabolic stress [20], reduced viability, or selective disruption of signalling remains to be determined. The observation that even viable DCs show impaired responsiveness underscores that ECT impacts more than simple cell survival. Future studies incorporating broader cytokine panels and downstream signalling analyses are required to fully delineate the molecular mechanisms underlying ECT-induced TLR pathway modulation.

The increase in TNFα secretion in response to CpG DNA following ECT at 800 V/cm, as well as the relative preservation of TNFα production in response to both LPS and R848 at 800 V/cm, is notable given the dual roles of TNFα in inflammation, tumour vasculature and immune activation [24]. Mature immunogenic DCs produce, among other cytokines, significant amounts of TNFα. Therefore, despite the absence of increased maturation marker of the DCs following 800 V/cm treatment, based on their TNFα secretion, the cells may be functionally mature. TNFα can promote anti-tumour responses through DC activation and T-cell stimulation but may also enhance tumour-supportive inflammation or regulatory T-cell induction depending on the context. TNFα also exhibits anti-vascular activity [25], which may potentiate or hinder ECT, depending on timing and local effects. This duality mirrors results from murine models in which TNFα administration enhanced ECT efficacy only when delivered prior, but not post, to treatment [26]. Whether endogenous TNFα released following ECT provides similar context-dependent effects remains to be determined.

Together, these results indicate that ECT imposes distinct immunomodulatory effects on DCs across different electric field strengths. Lower voltages (800 V/cm) preserve viability and maintain more functional responsiveness, whereas higher voltages (1000–1300 V/cm) induce phenotypic maturation but impair downstream signalling. These findings raise two translational opportunities: [1] low-intensity ECT may better support antigen-presenting cell function *in vivo*, potentially improving tumour antigen processing and T-cell priming; and [2] combining ECT with TLR-targeted immune agonists, especially TLR9 ligands, may compensate for suppressed pathways and enhance post-treatment immunogenicity. Moreover, as this study used naïve DCs *in vitro*, *in vivo* validation remains essential. Tumour-conditioned DCs are metabolically and phenotypically distinct from naïve cells [27], and the tumour microenvironment may amplify or dampen ECT-induced effects. Future studies, including murine ECT models and patient-derived analyses, will be necessary to determine whether the alterations in viability, TLR expression and cytokine responsiveness observed here translate into differential T-cell priming and anti-tumour immunity.

In conclusion, ECT exerts a complex, voltage-dependent influence on DC survival, phenotype, TLR biology and functional capacity. These findings strengthen the rationale for tailoring ECT parameters to optimise immune engagement and support the strategic pairing of ECT with immunostimulatory adjuvants such as CpG DNA [10]. By refining ECT protocols to better protect or activate DCs, it may be possible to enhance the therapy's immunogenic potential and improve clinical outcomes for cancer patients.

CRediT authorship contribution statement

Maura B. Bendix: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kevin J. Baker:** Investigation. **Patrick F. Forde:** Supervision, Funding acquisition, Conceptualization. **Aileen Houston:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Conceptualization. **Elizabeth Brint:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2026.109263.

Data availability

Data will be made available on request.

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