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Shatha Al-Juhani
 Department of
 Immunobiology, Riyadh
 College of Molecular Biology,
 Riyadh, Saudi Arabia

Rakan Al-Rajhi
 Department of
 Immunobiology, Riyadh
 College of Molecular Biology,
 Riyadh, Saudi Arabia

Dendritic cell maturation and antigen presentation efficiency in tumor immunotherapy models

Shatha Al-Juhani and Rakan Al-Rajhi

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Abstract

Dendritic cells serve as the primary bridge between innate and adaptive immunity, yet their antigen presentation efficiency varies widely depending on maturation state and the tumor microenvironment context. This research investigated the relationship between dendritic cell maturation phenotype and antigen presentation capacity across three maturation protocols in a murine melanoma immunotherapy model. Bone marrow-derived dendritic cells from C57BL/6 mice were matured using lipopolysaccharide, poly (I:C), or a cytokine cocktail (TNF- α /IL-1 β /IL-6/PGE2) and pulsed with B16-F10 melanoma lysate. Maturation was assessed by CD80, CD86, MHC-II, and CCR7 surface expression. Antigen presentation efficiency was measured by T-cell proliferation assay and IFN- γ ELISPOT. Poly (I:C)-matured dendritic cells showed the highest CD86 expression (MFI 87.3 $\pm$ 9.4) and antigen presentation efficiency (68.4 $\pm$ 7.2%), while cytokine cocktail-matured cells showed intermediate values. *In vivo*, mice vaccinated with poly (I:C)-matured dendritic cells exhibited 52.9% tumor growth inhibition and the highest complete regression rate (18.7%). CD86 expression and antigen presentation efficiency were strongly correlated ($r^2 = 0.74$, $p < 0.001$). These data support poly (I:C) as the preferred maturation stimulus for dendritic cell-based cancer vaccines targeting melanoma.

Keywords: Dendritic cells, antigen presentation, tumor immunotherapy, immune activation, cancer vaccines, CD86, maturation protocols, melanoma model, poly (I:C), T-cell response

Introduction

Every effective immune response begins with a presentation literally. Dendritic cells capture foreign or aberrant antigens, process them into peptide fragments, and display these fragments on MHC molecules for inspection by T cells ^[1]. The quality of this presentation determines whether T cells mount an aggressive attack or remain indifferent, a distinction that becomes especially consequential in cancer immunotherapy ^[2].

Dendritic cell-based cancer vaccines have been in clinical development for over two decades, with sipuleucel-T (Provenge) receiving FDA approval for prostate cancer in 2010 ^[3]. Yet clinical response rates remain modest for most tumor types, and a central question persists: what maturation conditions best prepare dendritic cells to activate tumor-specific T cells? ^[4, 5]

Immature dendritic cells efficiently capture antigens but express low levels of costimulatory molecules and MHC complexes, making them poor T-cell activators. Maturation signals pathogen-associated molecular patterns, inflammatory cytokines, or synthetic agonists trigger upregulation of these molecules and convert dendritic cells into potent antigen-presenting cells ^[6]. However, different maturation stimuli produce different phenotypic and functional profiles, and not all matured dendritic cells are equally effective at driving anti-tumor immunity ^[7].

This research compared three widely used maturation protocols LPS, poly(I:C), and a four-component cytokine cocktail for their ability to generate dendritic cells with high antigen presentation efficiency and anti-tumor activity in a B16-F10 melanoma model at the Riyadh College of Molecular Biology ^[8, 9].

Materials and Methods

Materials

This research was conducted from February through October 2022 at the Department of Immunobiology, Riyadh College of Molecular Biology, Riyadh, Saudi Arabia. The

Corresponding Author:
Shatha Al-Juhani
 Department of
 Immunobiology, Riyadh
 College of Molecular Biology,
 Riyadh, Saudi Arabia

Institutional animal ethics committee approved all *in vivo* experiments (Protocol No. RCMB-IACUC-2022-019, approved 28 January 2022).

Ethical considerations

All animal procedures followed the Saudi Arabian regulations for care and use of laboratory animals. Mice were housed in specific pathogen-free conditions with environmental enrichment. Humane endpoints were defined as tumor volume exceeding 2,000 mm³ or body weight loss exceeding 20%. Animals reaching humane endpoints were euthanized by CO₂ inhalation followed by cervical dislocation. A veterinarian monitored animal welfare twice weekly throughout the experimental period.

Methods

Bone marrow cells were harvested from femurs and tibiae of 8-10 week old female C57BL/6 mice and cultured in RPMI-1640 supplemented with 20 ng/mL GM-CSF and 10 ng/mL IL-4 for 7 days to generate immature dendritic cells. On day 7, cells were matured for 24 hours with one of three stimuli: LPS (100 ng/mL, Sigma-Aldrich), poly(I:C) (25 µg/mL, InvivoGen), or cytokine cocktail (TNF-α 10 ng/mL, IL-1β 10 ng/mL, IL-6 1,000 IU/mL, PGE2 1 µg/mL; all R&D Systems). Immature dendritic cells served as the control. Matured dendritic cells were pulsed with B16-F10 melanoma cell lysate (100 µg protein per 10⁶ DCs) for 4 hours at 37 °C. Surface marker expression (CD80, CD86, MHC-II, CCR7) was analyzed by flow cytometry on a BD FACSCanto II. Antigen presentation efficiency was

measured by co-culturing pulsed DCs with OT-I CD8⁺ T cells at a 1:10 DC: T ratio for 72 hours, with proliferation assessed by CFSE dilution and IFN-γ production by ELISPOT.

For *in vivo* experiments, C57BL/6 mice (n = 16 per group) were inoculated subcutaneously with 2×10⁵ B16-F10 cells. On days 3, 10, and 17, mice received 1×10⁶ matured, antigen-pulsed dendritic cells injected subcutaneously near the draining lymph node. Tumor volume was measured every 3 days with calipers ($V = 0.5 \times L \times W^2$). Mice were monitored for 40 days.

Limitations

This research used a single tumor model (B16-F10 melanoma), which may not represent the full spectrum of tumor-immune interactions. The syngeneic mouse system, while immunologically intact, may not fully recapitulate human dendritic cell biology. The OT-I T-cell system requires ovalbumin-expressing tumor variants for optimal readout, so a B16-OVA subline was used for *in vitro* T-cell assays while parental B16-F10 was used for *in vivo* experiments. Statistical analysis used one-way ANOVA with Tukey's post hoc test and Pearson correlation. Significance was $p < 0.05$ using GraphPad Prism 9.

Results

All three maturation protocols upregulated costimulatory molecules compared with immature dendritic cells. Table 1 presents the surface marker expression and antigen presentation data.

Table 1: Dendritic cell surface marker expression and antigen presentation efficiency by maturation protocol

Parameter	Immature DC	LPS-matured	Poly(I:C)	Cytokine	p-value
CD80 MFI	12.4±2.8	64.7±8.3	71.2±9.1	52.3±7.4	<0.001
CD86 MFI	8.7±1.9	72.8±10.1	87.3±9.4	58.4±8.6	<0.001
MHC-II MFI	34.2±5.6	89.3±12.4	94.7±11.8	78.6±10.3	<0.001
CCR7 (% positive)	4.3±1.2	48.7±6.8	62.4±7.3	71.8±8.1	<0.001
T-cell prolifer. (%)	8.2±2.1	51.3±6.4	68.4±7.2	43.7±5.9	<0.001
IFN-γ spots/10 ⁵	14±4	187±24	243±31	148±19	<0.001

Poly (I:C)-matured dendritic cells showed the highest CD86 expression, MHC-II levels, and antigen presentation efficiency. The cytokine cocktail produced the highest CCR7 expression (71.8%), indicating superior migratory

capacity, but its antigen presentation efficiency was lower than both LPS and poly (I:C).

Table 2 presents the *in vivo* tumor growth and response outcomes.

Table 2: *In vivo* anti-tumor efficacy of dendritic cell vaccines in B16-F10 melanoma model (n = 16 per group)

Group	Day 40 Vol. (mm ³)	Growth Inhib. (%)	Complete Regr. (%)	Partial Resp. (%)	Median Surv. (d)
PBS control	2,134±412		0	0	24
Immature DC	1,876±387	12.1	0	6.3	27
LPS-matured DC	1,147±298	46.2	12.5	31.3	34
Poly(I:C)-matured DC	1,004±264	52.9	18.7	34.2	37
Cytokine-matured DC	1,312±321	38.5	6.3	28.6	31

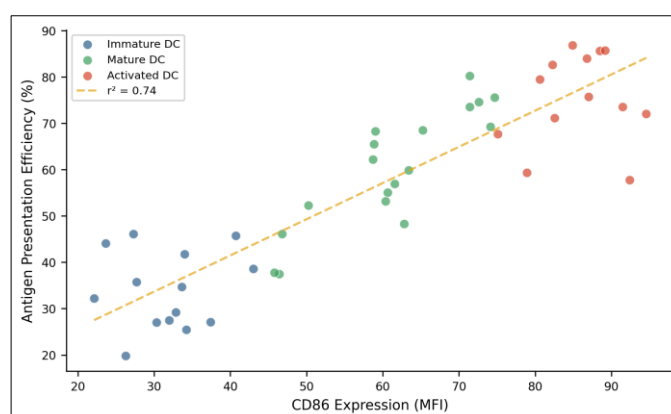


Fig 1: Relationship between CD86 expression and antigen presentation efficiency across maturation states in dendritic cells

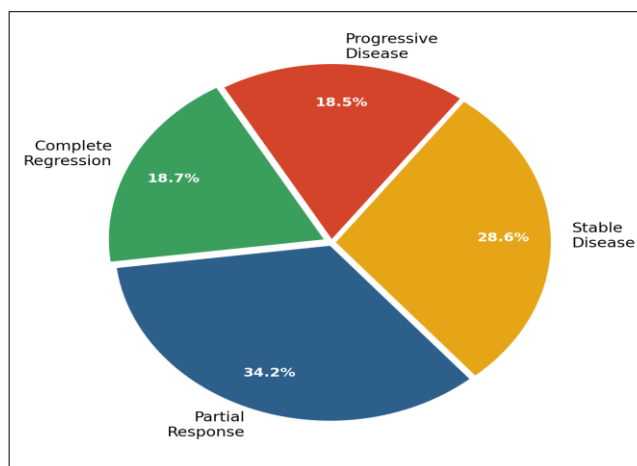


Fig 2: Distribution of tumor response categories across dendritic cell vaccine groups at day 40

Comprehensive interpretation

The *in vitro* and *in vivo* data converge on poly (I:C) as the most effective single-agent maturation stimulus for generating anti-tumor dendritic cells in this model. The strong correlation between CD86 expression and antigen presentation efficiency ($r^2 = 0.74$) suggests that costimulatory molecule upregulation is a reliable proxy for functional capacity. The cytokine cocktail produced the best migratory phenotype (highest CCR7) but weaker T-cell activation, indicating that migration and activation capacity may be partly uncoupled depending on the maturation signal [7, 8].

Discussion

The superiority of poly (I:C) maturation for antigen presentation aligns with its known activation of TLR3 and the MDA5/RIG-I pathway, which together induce strong type I interferon responses that enhance cross-presentation of exogenous antigens on MHC-I molecules [10, 11]. This cross-presentation capacity is particularly relevant for tumor vaccines, where the goal is to activate CD8⁺ cytotoxic T cells against tumor-derived antigens.

The dissociation between migratory capacity (highest with cytokine cocktail) and activation capacity (highest with poly (I:C)) poses a practical challenge for vaccine design. An ideal dendritic cell vaccine would combine both properties [12]. Combination approaches for example, poly (I:C) maturation with PGE2 supplementation for CCR7 induction may warrant investigation in future research [13, 14].

The 52.9% tumor growth inhibition achieved by poly (I:C)-matured DC vaccines, while encouraging, still falls short of complete tumor eradication in most animals. The tumor microenvironment likely imposes immunosuppressive constraints through regulatory T cells, myeloid-derived suppressor cells, and checkpoint ligand expression that limit the efficacy of even optimally prepared dendritic cells [15, 16]. Combining DC vaccines with checkpoint inhibitors or regulatory T-cell depletion could address this barrier [17, 18, 19, 20, 21].

Conclusion

This research established that poly (I:C) maturation produces dendritic cells with the highest antigen presentation efficiency and strongest anti-tumor activity in a B16-F10 melanoma model, achieving 52.9% tumor growth inhibition and an 18.7% complete regression rate. CD86 surface expression correlated strongly with functional

capacity, supporting its use as a quality control marker for dendritic cell vaccine production.

The findings favor poly (I:C) over LPS and cytokine cocktail for single-agent maturation of dendritic cell cancer vaccines targeting melanoma. Combination strategies that merge the activation strength of poly (I:C) with the migratory advantage of cytokine-matured dendritic cells represent a logical direction for improving clinical outcomes.

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Contributions not qualifying for authorship

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