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1,2-naphthoquinone enhances IFN-γ-induced MHC-I expression in dendritic cells, thereby inducing CD8 T cell activation

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ABSTRACT

Dendritic cells play a crucial role in immune responses by capturing pathogens and presenting antigens to T cells via major histocompatibility complex (MHC) molecules, thus triggering adaptive immune responses. 1,2-naphthoquinone (1,2-NQ), a quinone found in diesel exhaust and cigarette smoke, has various physiological functions. In this study, we investigated the effect of 1,2-NQ on the expression of antigen presentation-related molecules in the dendritic cell line DC2.4. The results revealed that 1,2-NQ enhanced the IFN-γ-induced upregulation of MHC-I expression at the transcriptional level. Moreover, it upregulated the expression of NLRC5, a transcriptional activator of MHC-I. 1,2-NQ is a reactive oxygen species (ROS) producing reagent. The 1,2-NQ-induced upregulation of MHC-I expression and downregulation of MHC-II expression were abolished by the ROS scavenger N-acetylcysteine. Similar effects on MHC expression were also observed with ROS-inducing reagents, such as paraquat and diethyl maleate. In addition, dendritic cells stimulated with 1,2-NQ exhibited enhanced efficacy in CD8 T cell activation, which was accompanied by increased IFN-γ production by T cells. These findings demonstrate that 1,2-NQ enhances the IFN-γ-induced activation of dendritic cells and promotes the activation of CD8 T cells.

1. Introduction

Dendritic cells play a crucial role in immune responses; they capture pathogens, present them to T cells via major histocompatibility complexes (MHC), and activate adaptive immune responses to eliminate pathogens. MHC-I and MHC-II present antigens to CD8 T cells (cytotoxic T cells) and CD4 T cells (helper T cells), respectively, and induce T cell activation [1–3]. Stimulation by signals such as Toll-like receptor (TLR) family members, which are bacterial component receptors, or cytokines like IFN-γ is typically necessary for changes in the expression of antigen presentation-related molecules on the dendritic cell surface and cytokine production during the induction of dendritic cell-mediated immune responses [4–6]. IFN-γ, mainly produced by activated Th1 cells and natural killer (NK) cells, presents a stimulus that induces dendritic cell activation. IFN-γ enhances the expression of antigen-presenting factors, such as MHC-I and MHC-II on dendritic cells, thereby promoting the elimination of infected bacteria and tumors [7].

Quinones are a group of dicarbonyl compounds in which two C-H groups in an aromatic hydrocarbon skeleton are replaced by C=O groups. Quinones exhibit beneficial as well as harmful physiological effects through several mechanisms, including the production of reactive oxygen species (ROS) and protein modification [8]. Vitamin K, phylloquinone, menaquinone, and menadione are involved in blood coagulation and bone formation. These quinones have also been reported to possess anti-inflammatory effects, such as suppression of inflammatory cytokine production [9]. Several quinone compounds have been used as anticancer drugs owing to their ability to induce apoptosis in tumor cells via ROS mediation [10].

Naphthoquinones (NQ), such as 1,2-NQ and 1,4-NQ, are oxidation products of naphthalene, which are common in exhaust gases and cigarette smoke. NQs exhibit various physiological effects. At high concentrations, NQs cause skin and respiratory irritation, induce inflammation, and lead to carcinogenesis via various mechanisms, including ROS production, protein modification, and DNA damage

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Abbreviations: APC, allophycocyanin; BQ, benzoquinone; CIITA, class II major histocompatibility complex transactivator; DEM, diethyl maleate; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; MHC, major histocompatibility complex; JAK, janus kinase; MD, menadione; NAC, N-acetylcysteine; NQ, napthoquinone; NLRC5, NOD-, LRR- and CARD-containing 5; OVA, ovalbumin; PE, phycoerythrin; RT-PCR, reverse transcription-polymerase chain reaction; ROS, reactive oxygen species; STAT, signal transducers and activator of transcription; TLR, toll like receptor.

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Table 1
List of primers used.

Gene	Forward (5′ to 3′)	Reverse (5′ to 3′)
Gapdh	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG
H2-K1	ATCTGTGGTGGTGCCTCTTG	TCCACCTGTGTTTCTCCTTCTC
Nlrc5	TTGCCTACTATGGGGAGCCT	AGCTCCACAAGACTCAGCAC
H2-Ab1	GGCGAGTGCTACTTCACCAA	GGGTTACAGCAGTAGAGGGA
Ciita	AAGGATCTTCCTGCCATCCG	TTAGGAGGGACTTGGCTAGGG

[11–13]. Contrastingly, NQ-derived compounds have been found to possess beneficial effects such as antibacterial, wound healing, anti-tumor, anti-inflammatory, and neuroprotective activities, suggesting their potential effectiveness as therapeutic agents [14].

However, the mechanism underlying the effect of NQs on the immune responses associated with antigen presentation by dendritic cells remains unclear. Hence, in this study, we investigated the effects of 1,2-NQ on the antigen-presenting activity in dendritic cells.

2. Materials and methods

2.1. Materials

1,4-naphthoquinone (1,4-NQ), p-benzoquinone (p-BQ), paraquat (PQ), and diethyl maleate (DEM) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Menadione (MD), H_2O_2, N-acetylcysteine (NAC), and anti-mouse GAPDH antibody (5A12) were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan), 1,2-NQ from Sigma-Aldrich (St. Louis, MO, USA), NucleoSpin RNA kit, PrimeScript RT reagent kit from Takara Bio (Shiga, Japan), and KOD SYBR qPCR Mix from Toyobo (Osaka, Japan). The ELISA kit for mouse IFN-γ and the following antibodies: recombinant mouse IFN-γ, PE-labeled anti-mouse MHC-I (clone: AF6-88.5), FITC-labeled anti-mouse MHC-II (clone: M5/114.15.2), APC-labeled anti-mouse MHC-II (clone: AF6-120.1), FITC-labeled anti-mouse CD80 (16-10A1), FITC-labeled anti-mouse CD86 (GL-1), PE-labeled anti-mouse PD-L1 (10F.9G2), FITC-labeled anti-mouse CD3ε (145-2C11), PerCP/cyanine5.5-labeled anti-mouse CD4 (GK1.5), PE/cyanine7-labeled anti-mouse CD11c (N418), APC-labeled anti-mouse CD8 (53-6.7), PE-labeled anti-mouse CD69 (H1.2F3), anti-pSTAT1 (clone: A15158B), and anti-total STAT1 (clone: 10C4B40), were purchased from BioLegend (San Diego, CA, USA). All other chemicals were commercial products of reagent grade.

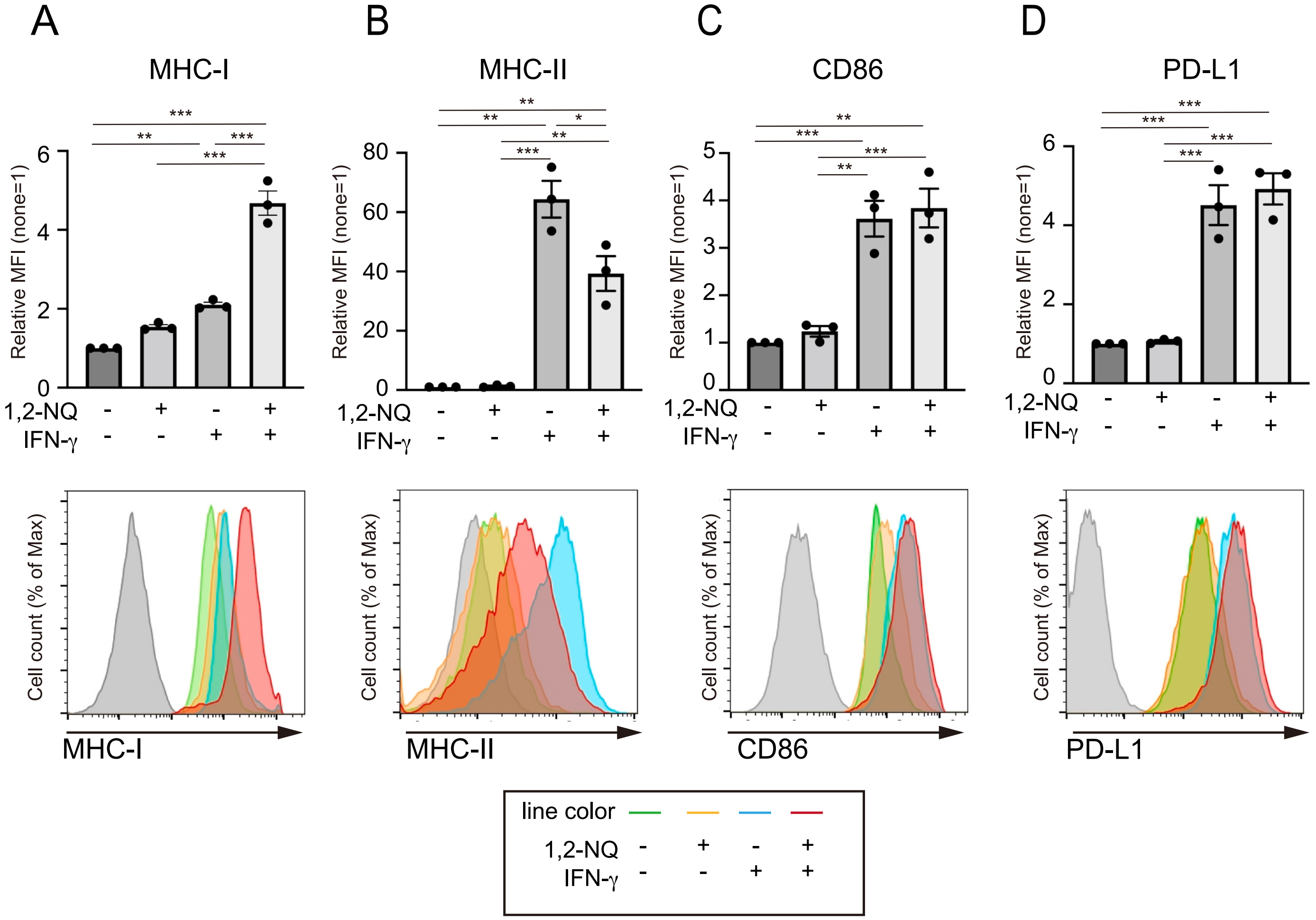

Fig. 1. Effects of 1,2-NQ on the cell surface expression of antigen-presentation-related molecules
DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,2-NQ (8.3 μM) for 24 h at 37 °C. The cell surface expression of MHC-I (A), MHC-II (B), CD86 (C), and PD-L1 (D) was analyzed using flow cytometry. Representative histograms are shown below the graphs. The experimental conditions corresponding to the line colors of the histograms are shown in the box below (light gray fill, control IgG). The graphs present the relative mean fluorescence intensity (no stimulation = 1). The. values are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between the groups were determined using Tukey's multiple comparison test. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

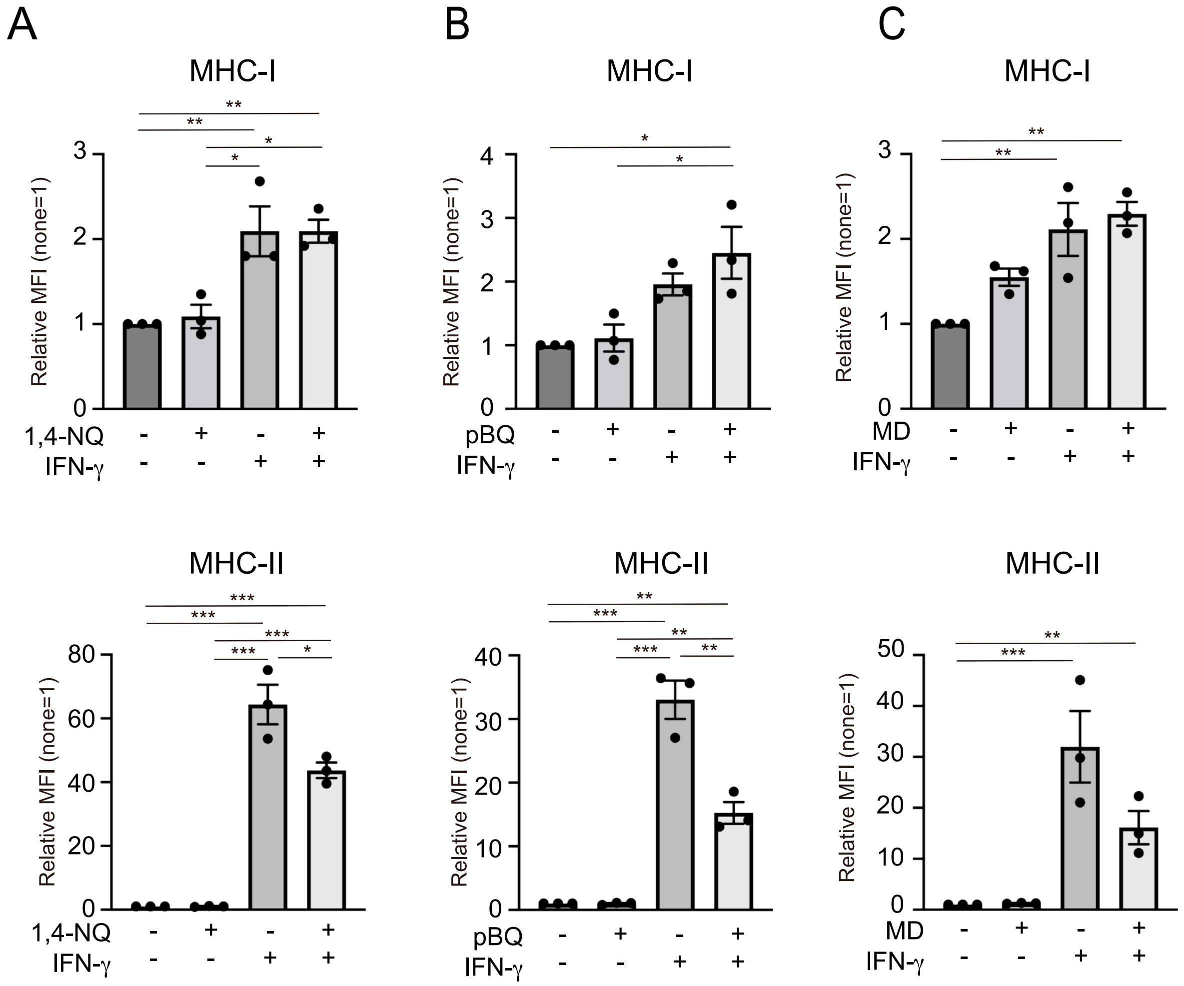

Fig. 2. Effects of 1,2-NQ on the cell surface-specific expression of antigen-presentation-related molecules

DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,4-NQ (0.5 μM) (A), pBQ (10 μM) (B), or MD (10 μM) (C) for 24 h at 37 °C. The cell surface expression of MHC-I and MHC-II was analyzed using flow cytometry. The graphs present the relative mean fluorescence intensity (no stimulation = 1). The values are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between groups were determined using Tukey's multiple comparison test. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

2.2. *Mice*

Male BALB/c mice (5–8 weeks old) were obtained from Japan SLC (Hamamatsu, Japan) and housed in an animal facility at Okayama University. This study was approved by the Committee on Animal Experiments, Okayama University (OKU-2022127, OKU-2023354).

2.3. *Cell culture*

DC2.4 cells, a mouse dendritic cell line, was kindly provided by Dr. Kenneth Rock (Dana Farber Cancer Institute, Boston, MA) [15]. DC2.4 cells were cultured in RPMI-1640 medium supplemented with 10 % FBS, 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 50 μM 2-mercaptoethanol at 37 °C in the presence of 5 % CO_2. To avoid mycoplasma contamination and maintain authenticity, the cell lines were routinely tested using PCR analyses.

2.4. *Flow cytometry analysis*

Cells were harvested and washed with FACS-SM (PBS(-) containing 2 % FBS, 0.05 % NaN_3). Subsequently, the cells were incubated with fluorescent dye-conjugated antibodies in FACS-SM on ice for 30 min. The cells were washed three times and analyzed using a flow cytometer (Gallios: Beckman Coulter).

2.5. *Quantitative RT-PCR*

Total RNA was extracted using the NucleoSpin RNA kit and reverse transcribed into cDNA using the PrimeScript RT reagent kit. PCR was performed using KOD SYBR qPCR mix and primers for the target genes. The primer pairs for the target genes, MHC-I (*H2-K1*), MHC-II beta chain (*H2-Ab1*), NLRC5 (*Nlrc5*), CIITA (*Ciita*), and GAPDH (*Gapdh*) are shown in Table 1. The results were normalized to GAPDH expression.

2.6. *Immunoblotting*

Immunoblot analyses were performed following a previously described method [16]. Samples were subjected to sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred onto a polyvinylidene difluoride membrane. Primary antibodies (anti-pSTAT1, anti-total STAT1, or anti-GAPDH) and horseradish peroxidase-conjugated anti-mouse IgG, used as secondary antibody, were used to analyze the expression of pSTAT1, total STAT1, and GAPDH. Membranes were re-probed by treating with stripping buffer (1 % SDS, 20 mM glycine, pH 2.0) at room temperature for 30 min. Bands were quantified using ImageJ software.

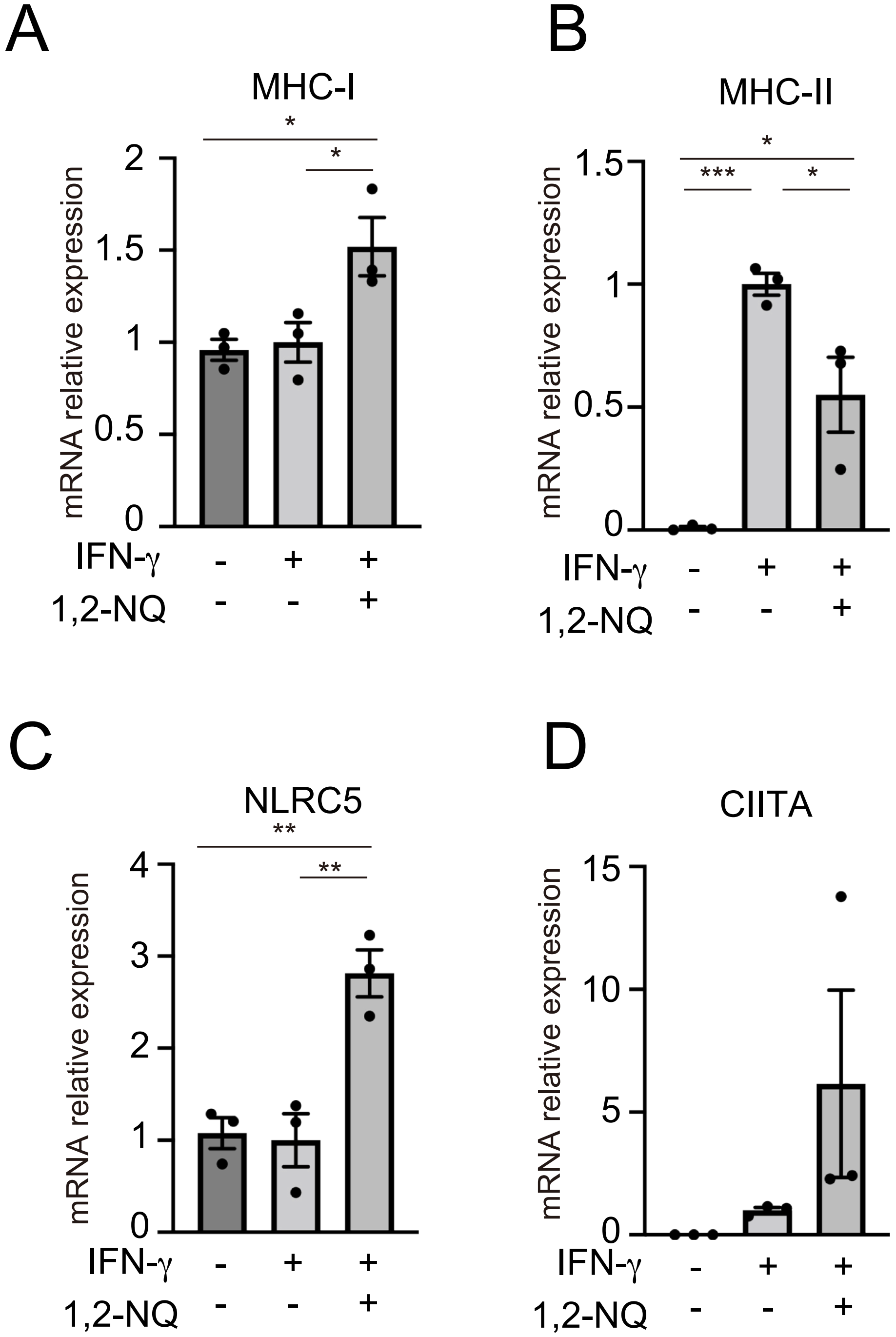

Fig. 3. Effects of 1,2-NQ on the mRNA expression of MHC and regulation factors
DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of 1,2-NQ (8.3 μM) for 24 h at 37 °C. The mRNA expression levels of MHC-I (A), MHC-II (B), NLRC5 (C), CIITA (D), and GAPDH were analyzed using qPCR. Data are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between the groups were determined using Tukey's multiple comparison test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

2.7. Mixed lymphocyte reaction (MLR)

An MLR assay was performed following a previously described protocol [17]. For this analysis, lymph nodes were collected from BALB/c mice; a single-cell suspension was prepared by crushing the lymph nodes and removing debris using a cell strainer. DC2.4 cells (2×10^4 cells) were cocultured with lymph node cells (4×10^5 cells) for 72 h. The culture supernatants were harvested and the amounts of IFN-γ were analyzed via ELISA. To analyze the T cell activation, the cells were harvested, and the cell surface expression levels of CD69, CD3ε, CD4 or CD8, and CD11c were analyzed using a flow cytometer.

2.8. Statistical analysis

The experiments were independently performed at least three times. The number of trials is indicated as "n" in the figure legends. Results are presented as mean ± SEM. Student's t-test was used to compare data between two groups. For comparisons among three or more groups, multiple comparisons were performed using one-way ANOVA with Tukey's post-hoc test or Dunnett's multiple comparison test. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered statistically significant.

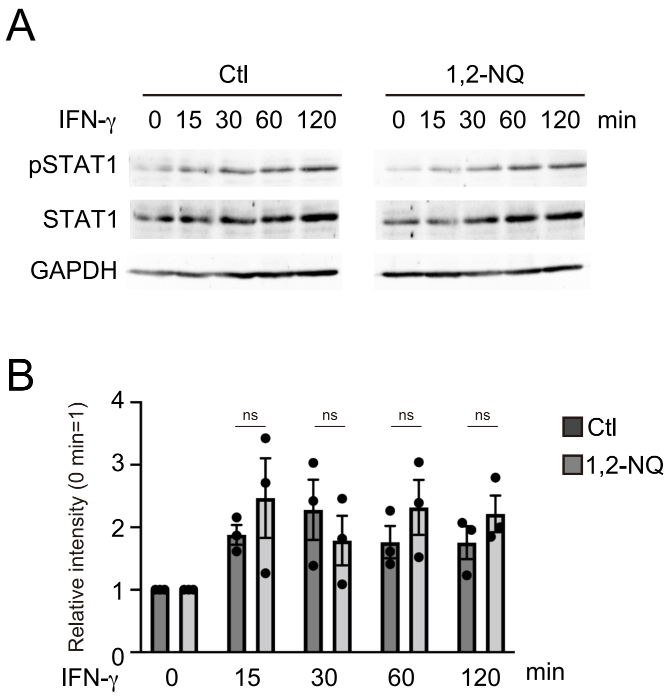

Fig. 4. Effects of 1,2-NQ on IFN-γ-induced STAT1 phosphorylation
(A) DC2.4 cells were pretreated with 1,2-NQ (8.3 μM) for 30 min, then the cells were stimulated with IFN-γ (20 ng/mL) for 0, 15, 30, 60 and, 120 min. The levels of pSTAT1, STAT, and GAPDH were detected through immunoblotting analysis using specific antibodies. (B) The graph presents the quantification of bands (0 min = 1). Data are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between the control and 1,2-NQ were determined using the student's t-test. ns: not significant.

3. Results

3.1. Effects of quinone compounds on the cell surface expression of antigen-presentation-related molecules

NQs exhibit cytotoxicity at high concentrations [13,18]. Therefore, the effects of 1,2-NQ and 1,4-NQ on the viability of DC2.4 cells was examined. 1,2-NQ decreased cell viability at 20 μM (Fig. S1A), whereas 1,4-NQ caused a reduction in viability at concentrations above 5 μM (Fig. S1B). In this study, concentrations that did not affect cell viability were used. To analyze the effect of 1,2-NQ on the antigen-presenting function of dendritic cells, DC2.4 cells were stimulated with IFN-γ in the presence of 1,2-NQ. The effects on antigen presentation-related molecules MHC-I, MHC-II, CD86, and PD-L1 were investigated. The expression of these antigen presentation-related molecules was not affected by 1,2-NQ alone (Fig. 1A–D). However, in the presence of 1,2-NQ, IFN-γ-induced expression of MHC-I on the cell surface was enhanced, whereas, the IFN-γ-induced upregulation of MHC-II expression was suppressed (Fig. 1A and B). The presence of 1,2-NQ did not affect the expression of CD86 or PD-L1 (Fig. 1C and D). To determine the effective concentration of 12-NQ that enhances MHC-I expression, we evaluated various concentrations and found that a significant upregulation was observed at concentrations of 1 μM or higher (Fig. S2). To determine whether the effect of 1,2-NQ is specific to DC2.4 cells, we examined the effects of 1,2-NQ on bone marrow-derived dendritic cells (BMDCs) and the melanoma cell line B16F10. In BMDCs, 1,2-NQ enhanced the IFN-γ-induced increase in MHC-I expression (Fig. S3A), whereas no significant suppression was observed in the upregulation of MHC-II expression (Fig. S3B). We examined the presentation of exogenously acquired ovalbumin (OVA) protein-derived peptide (SIINFEKL) on MHC-I using a specific antibody, and found that the expression level of MHC-I/OVA complexes was also enhanced by 1,2-NQ (Fig. S3C). Similarly, in the melanoma cell line B16F10, IFN-γ stimulation led to an increase in MHC-I expression, which was further enhanced by 1,2-NQ (Fig. S4). We examined the effects of 1,4-NQ, p-benzoquinone (pBQ), and menadione (MD) on MHC-I and MHC-II expression. These quinones did not enhance MHC-I expression; however, they suppressed MHC-II expression, which was comparable to the effects of 1,2-NQ (Fig. 2A–C).

3.2. Effects of 1,2-NQ on the mRNA expression of MHC and transcriptional regulatory factors

Quantitative RT-PCR analysis revealed the effects of 1,2-NQ on MHC-I and MHC-II expression at the mRNA level. After DC2.4 cells were stimulated with IFN-γ in the presence of 1,2-NQ, the mRNA expression of MHC-I was enhanced, while MHC-II expression was suppressed, demonstrating changes similar to those observed on the cell surface (Fig. 3A and B). The expression analysis of NLRC5 and CIITA, the transcriptional regulatory factors for MHC-I and MHC-II, respectively [19,20] revealed that NLRC5 expression was enhanced by 1,2-NQ, similarly to the MHC-I expression profile (Fig. 3C). In BMDCs an increase in NLRC5 expression was observed in the presence of 1,2-NQ (Fig. S5). Moreover, the expression of CIITA tended to be enhanced in the presence of 1,2-NQ (Fig. 3D). IFN-γ induces the activation of the JAK1/2 and STAT1 signaling pathways, leading to the induction of MHC-I and MHC-II expression through transcriptional activation [19,21]. Further analyses indicated that 1,2-NQ did not alter IFN-γ-induced STAT1 phosphorylation in DC2.4 (Fig. 4A and B).

3.3. Association of ROS with the effects of 1,2-NQ on MHC expression

Quinones induce oxidative stress via ROS production [22]. Therefore, we examined the effects of NAC, a ROS scavenger, on the effects of 1,2-NQ. NAC-treated DC2.4 cells were stimulated with IFN-γ in the presence of 1,2-NQ, followed by the analyses of cell surface MHC-I and MHC-II expression profiles. The results revealed that the NAC treatment attenuated the effects of 1,2-NQ on MHC-I and MHC-II expression (Fig. 5A). We investigated the effects of H_2O_2, PQ, and DEM, reagents known to induce intracellular ROS production [23,24], on the IFN-γ-induced effects on MHC-I and MHC-II expression. PQ and DEM enhanced the IFN-γ-induced upregulation of MHC-I expression, while suppressing the upregulation of MHC-II expression (Fig. 5B). These effects were not observed with H_2O_2 (Fig. 5B). These results suggest that ROS are involved in the impact of 1,2-NQ on MHC expression.

3.4. Effect of 1,2-NQ on T cell activation by dendritic cells

Next, we demonstrated the effects of 1,2-NQ on the antigen-presenting functions of dendritic cells. The MLR assay revealed T cell activation via antigen presentation by 1,2-NQ-stimulated dendritic cells, which reflects an allo-MHC-I- or allo-MHC-II-dependent T-cell activation response and antigen presentation by MHC-I or MHC-II [25]. The activation of CD4 and CD8 T cells was analyzed by examining the induction of cell surface CD69 expression, a T cell activation marker, and IFN-γ production. An increase in activated CD8 T cells was observed in dendritic cells stimulated with IFN-γ in the presence of 1,2-NQ, whereas, no difference was detected in CD4 T cell activation (Fig. 6A). Additionally, an increase in IFN-γ production was observed (Fig. 6B).

4. Discussion

This study demonstrates the effects of quinone compounds on dendritic cell function analyzed in terms of their impact on the cell surface-specific expression of antigen presentation-related molecules. The results revealed that 1,2-NQ enhances the IFN-γ-induced upregulation in the expression of MHC-I on the surface of dendritic cells. On the other hand, 1,2-NQ suppressed the increase in cell surface MHC-II expression induced by IFN-γ stimulation. Furthermore, dendritic cells stimulated

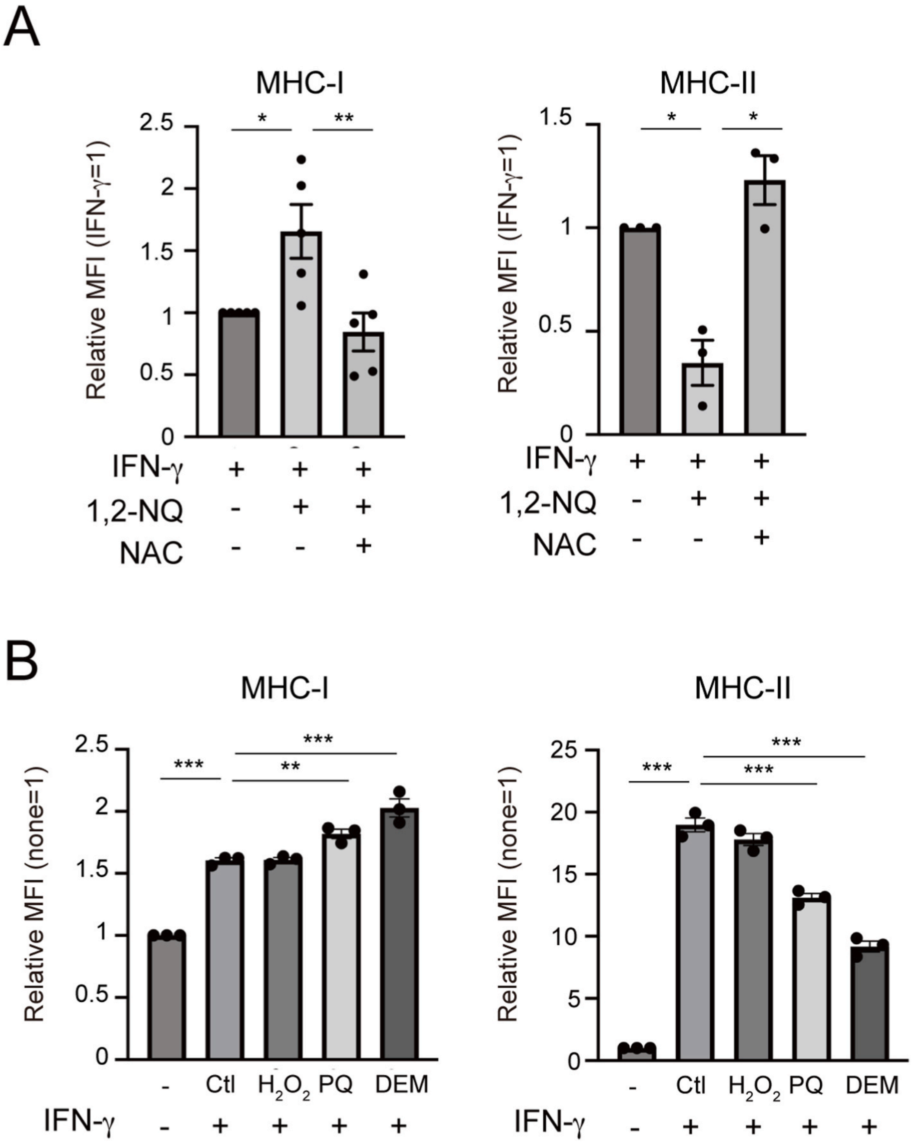

Fig. 5. Involvement of ROS in the effect of 1,2-NQ on MHC expression
(A) DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL), 1,2-NQ (8.3 μM), and NAC (1 mM) for 24 h at 37 °C. (B) DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL) in the presence of H_2O_2 (100 μM), paraquat (PQ) (10 μM) or diethyl malate (DEM) (30 μM) for 24 h at 37 °C. The cell surface expression of MHC-I and MHC-II was analyzed using flow cytometry. The graphs present the relative means of fluorescence intensity (no stimulation = 1). The values are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between groups were determined using Tukey's multiple comparison test. $^*p < 0.05$, and $^{**}p < 0.01$. ns: not significant.

with IFN-γ in the presence of 1,2-NQ strongly induced the activation of CD8 T cells and increased IFN-γ production, which suggests that 1,2-NQ enhances the effect of IFN-γ and promotes CD8 T cell activation via MHC-I.

1,2-NQ upregulates the expression of MHC-I and NLRC5 mRNAs. Therefore, we suggest that 1,2-NQ activates IFN-γ-induced MHC-I expression by upregulating the expression of NLRC5. IFN-γ binds to the IFN-γ receptor (IFNGR1/IFNGR2) and activates STAT1 via JAK1/2 [26,27]. Previous reports indicate that IFN-γ induces NLRC5 expression through the JAK/STAT pathway [28,29]. Therefore, we analyzed the effects of 1,2-NQ on the JAK/STAT pathway. However, the lack of change in the IFN-γ-mediated induction of STAT1 phosphorylation in the presence of 1,2-NQ suggests that the increase in expression of NLRC5 by 1,2-NQ occurs downstream of the STAT1 activation. 1,2-NQ suppressed IFN-γ-stimulated upregulation of MHC-II expression at both protein and mRNA levels. Although 1,2-NQ increased CIITA mRNA expression, it suppressed MHC-II mRNA expression. 1,2-NQ may suppress MHC-II by inhibiting the transcriptional regulation of MHC-II, acting downstream of CIITA expression, or the effect of 1,2-NQ may lead to the activation of transcriptional repressors of MHC-II. Furthermore, the suppression of MHC-II expression induced by 1,4-NQ, pBQ, and MD indicates a common effect exhibited by these quinones. The effects observed in this study may be due to 1,2-NQ acting on factors involved in the IFN-γ signaling pathway, leading to its effects specifically upon IFN-γ stimulation. The effects of 1,2-NQ observed in DC2.4 cells were also seen in BMDCs, where an enhancement of MHC-I expression was detected. However, no significant change in MHC-II expression was observed. This may be due to differences in the sensitivity or responsiveness to 1,2-NQ between different cell types.

Quinone compounds induce ROS production [22]. When DC2.4 cells were treated with the ROS scavenger NAC, the influence of 1,2-NQ on MHC-I and MHC-II expression was suppressed, suggesting that ROS production is involved in the effects of 1,2-NQ. The enhancement of IFN-γ-induced MHC-I expression and suppression of MHC-II expression

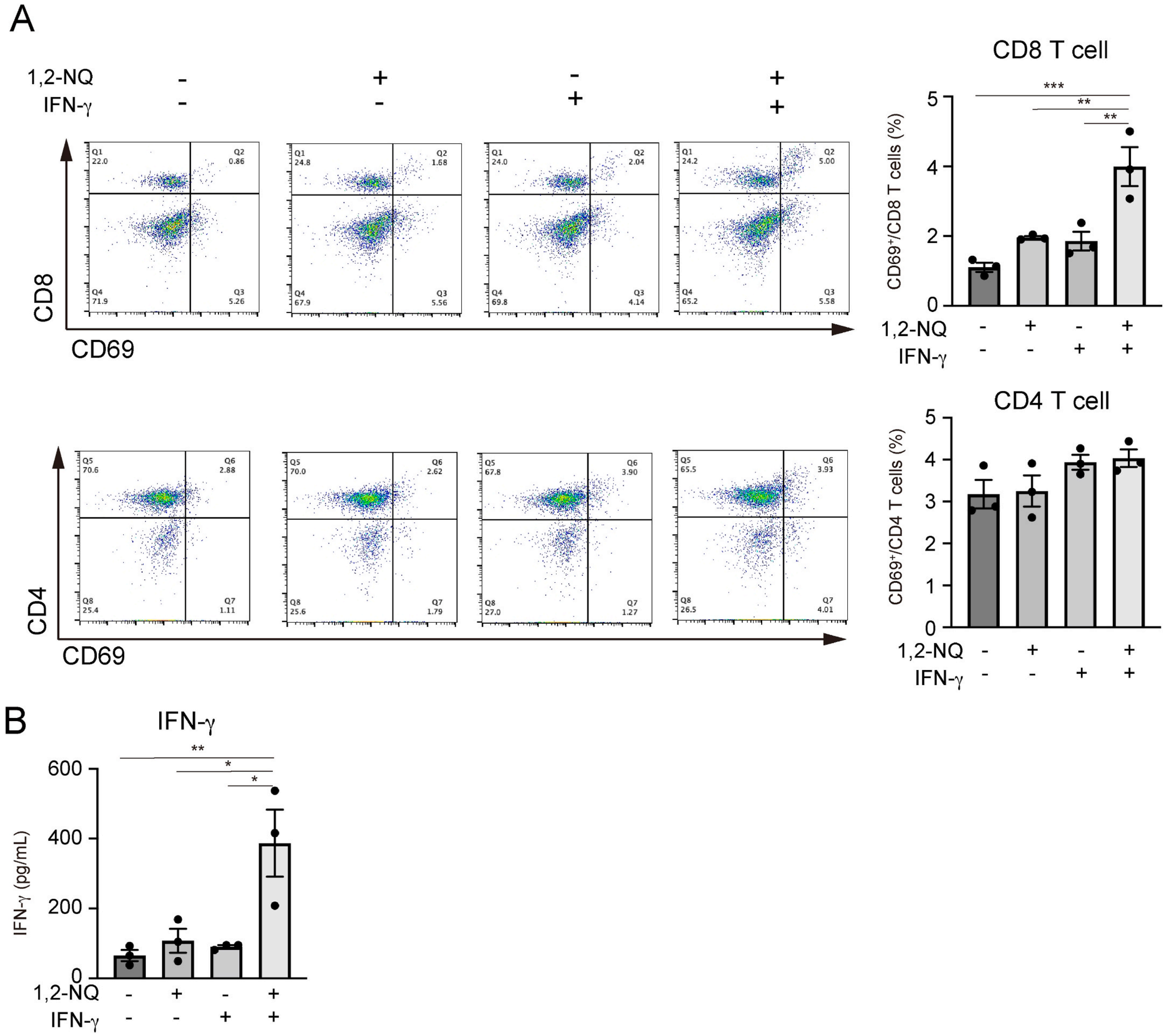

Fig. 6. Effect of 1,2-NQ on T cell activation by dendritic cells

DC2.4 cells were incubated with (+) or without (-) IFN-γ (20 ng/mL), and 1,2-NQ (8.3 μM) for 24 h at 37 °C. The DC2.4 cells and lymph node cells (DC: LN cell = 1:20) were cocultured for 72 h at 37 °C for the mixed lymphocyte reaction (MLR) assay. (A) Cell surface CD69 expression levels on CD4 T and CD8 T cells were analyzed using flow cytometry. After gating T cells with CD3, CD4 T cells and CD8 T cells were gated using CD4 and CD8, respectively. The ratios of $CD69^+$ in CD4 T or CD8 T cells (the proportion in the upper-right quadrant of the dot blot) are shown in the graph. (B) The amount of IFN-γ in the coculture medium was measured through ELISA. The values are expressed as the average ± SEM (n = 3, from three independent experiments). Statistical differences between the groups were determined using Tukey's multiple comparison test. $^*p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

were observed with ROS-inducing reagents such as PQ and DEM suggesting that the effects of 1,2-NQ are likely due to ROS production. However, similar effects were not observed with H_2O_2. PQ induces the production of superoxide, which is subsequently converted to H_2O_2 within cells [23]. DEM depletes glutathione, thereby disrupting intracellular ROS metabolism and leading to the accumulation of intracellular ROS [24]. Differences in the mechanisms of ROS production may result in variations in the site and duration of ROS generation, which may account for the differences in their effects. NQs exert their physiological effects through various mechanisms [12]. 1,2-NQ forms covalent bonds by nucleophilically reacting with other molecules and readily binds to macromolecules such as DNA and proteins. Proteins form irreversible thioether adducts with the thiol group of cysteine residues in a reaction known as arylation/alkylation [13,22]. 1,2-NQ activates the Akt signaling pathway downstream of the epidermal growth factor receptor (EGFR) by forming an N-allyl linkage with EGFR inducing tumor cell proliferation [30]. 1,2-NQ was reported to modulate immune responses through direct binding to IKK (inhibitor of IκB kinase) and inhibited transcription of cytokine gene, thereby suppressing NF-κB activity [31]. Furthermore, 1,2-NQ binds to protein tyrosine phosphatase PTP1B, inhibits its activity, and enhances EGF signaling [32]. These effects may also be related to the actions observed on MHC-I and MHC-II.

In the immune response, dendritic cells present antigens to CD8 T cells via MHC-I, whereas MHC-II mediates antigens presentation to CD4 T cells. IFN-γ increases the cell surface expression of both MHC-I and

MHC-II [5,33]. When IFN-γ-induced-MHC-I expression was upregulated by 1,2-NQ in DC2.4 cells, activation of CD8 T cells was enhanced through antigen presentation. This result is consistent with the function of MHC-I in presenting antigens to CD8 T cells. In contrast, when 1,2-NQ suppressed the IFN-γ-induced MHC-II expression, activation of CD4 T cells did not decreased. This is potentially attributed to the fact that during 1,2-NQ-mediated downregulation of MHC-II, its expression level is sufficiently high to activate CD4 T cells. In this study, we examined T cell activation using the MLR and observed enhanced activation of CD8 T cells along with increased IFN-γ production in the co-culture supernatant. Based on the result that the expression level of OVA-bound MHC-I was also enhanced in IFN-stimulated dendritic cells in the presence of 1,2-NQ, it is possible that antigen presentation to CD8 T cells is enhanced. It is likely that the enhanced production of IFN-γ originates from the activated CD8 T cells, but further investigation is required to determine which specific cells produce IFN-γ at higher levels. Additionally, the MLR response is a specific activation of CD8 T cells and CD4 T cells via MHC-I and MHC-II, respectively, but it is not an antigen-specific response involving the binding of antigens to MHC molecules. Therefore, to determine whether the enhancement of MHC-I observed in this experiment occurs with antigens internalized through cross-presentation or with endogenous antigens, experiments using antigen-specific T cells are necessary. This is a limitation of the current study and an area for future investigation.

The MHC-I and MHC-II-mediated presentation of tumor antigens to T cells by dendritic cells is crucial for antitumor immune responses leading to tumor elimination [34,35]. MHC-I activates CD8 T cells [3], which directly kill tumor cells; hence, increased MHC-I expression and activation of CD8 T cells by 1,2-NQ may lead to activate tumor elimination capacity. In addition, activated CD8 T cells recognize and kill tumor cells which present tumor antigens on MHC-I molecules [34]. Reduced MHC-I expression has been observed in certain tumor cells that escape CD8 T cell-mediated immune response [36]. In this study, we demonstrated that the IFN-γ-induced upregulation of MHC-I expression in the melanoma cell line B16F10 was enhanced by 1,2-NQ. Therefore, 1,2-NQ may induce the enhancement of antitumor immune responses by acting on cancer cells. Further elucidation of the mechanism underlying 1,2-NQ-induced MHC-I expression can help identify the regulatory factors for MHC-I expression, which can be applied to antitumor therapeutic methods for the appropriate activation of CD8 T cells.

The titration experiment provides a quantitative comparison of 1,2-NQ concentrations *in vitro* with estimated exposure levels *in vivo*. The concentrations used in our *in vitro* assays (1–10 μM) are higher than typical environmental exposure levels [37]. However, such concentrations are commonly used to reveal mechanistic effects [30,32]. These findings indicate that while the concentrations used in this study may exceed physiological exposure, they enable the identification of potential cellular targets and pathways affected by 1,2-NQ, which could also operate under chronic low-level exposure *in vivo*. Furthermore, cigarette smoke directly reaches the lungs during smoking, potentially leading to locally high concentrations of 1,2-NQ in lung tissue. To clarify the physiological relevance of our findings, it is necessary to investigate the effects of 1,2-NQ on immune responses *in vivo* using physiologically relevant concentrations of 1,2-NQ in future studies.

CRediT authorship contribution statement

Kazuyuki Furuta: Writing – original draft, Supervision, Investigation, Funding acquisition, Conceptualization. **Kanon Miyazato:** Writing – original draft, Investigation, Conceptualization. **Kai Kobata:** Investigation. **Kazuya Ishikawa:** Writing – review & editing, Conceptualization. **Chikara Kaito:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Data availability statement

The data supporting the findings of this study are available within the article.

Funding sources

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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.bbrc.2025.152453.

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