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Enhancement of T cell-mediated immune responses to whole inactivated influenza virus by chloroquine treatment *in vivo*

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ABSTRACT

Current influenza vaccines induce poor cross-reactive CD8+ T cell responses. Cellular immunity is generally specific for epitopes that are remarkably conserved among different subtypes, suggesting that strategies to improve the cross-presentation of viral antigens by dendritic cells (DC) could elicit a broadly protective immune response. Previous studies have shown that limited proteolysis within the endocytic pathway can favorably influence antigen processing and thus immune responses. Herein, we demonstrate that chloroquine improves the cross-presentation of non-replicating influenza virus *in vitro* and T cell responses in mice following a single administration of inactivated HI-X31 virus. CD8+ T cells were also recruited to lymph nodes draining the site of infection and able to reduce viral load following pulmonary challenge with the heterologous PR8 virus. These findings may have implications for vaccination strategies aimed at improving the cross-presentation capacity of DCs and thus the size of effector and memory CD8+ T cells against influenza vaccines.

1. Introduction

Current strategies for influenza vaccination focus on the use of inactivated virus to induce neutralizing antibodies, which are strain-specific and protective against homologous virus infection yet less effective against heterologous infection [1,2]. Several studies have shown that natural infection with influenza virus elicits T cell responses, which are cross-protective against different influenza subtypes [3–6]. Although cytolytic T lymphocytes (CTLs) cannot induce sterilizing immunity, the accumulation of a large number of them at sites of infection could reduce greatly the viral load in infected individuals by producing antiviral cytokines and killing infected cells. Thus improving the recall and induction of CTLs, which are poorly induced by vaccines based on whole inactivated influenza virus, could provide a great advantage in protecting against influenza. The various strategies for improving the induction of CTLs include targeting vaccines to antigen-presenting cells (APC) by opsonizing them with the corresponding IgG molecules and the use of different adjuvant formulations [7–11].

Chloroquine is one of the most widely used antimalarial drugs, and it has also been considered for treating viral infections and cancer [12–16]. Recently, chloroquine treatment was shown to improve the cross-presentation of soluble antigens in maturing dendritic cells (DC), which are otherwise susceptible to rapid endosomal protease degradation sustained by the acidic pH [17–19]. In particular, the administration of a booster dose of anti-hepatitis B vaccine, in association with a short-course of chloroquine treatment *in vivo*, was shown to significantly enhance the recall of memory CTLs in healthy individuals, compared to individuals who had not received chloroquine [17]. In the present study, we investigated whether chloroquine treatment could improve the cross-presentation of non-replicating influenza virus both *in vitro* and *in vivo* and thus increase the size of effector and memory T cells in mice vaccinated against influenza.

2. Materials and methods

2.1. Mice and viruses

C57BL/6 mice and OT-I and OT-II TCR-transgenic mice were obtained from Charles River, Italy. All of the mice were maintained in accordance with institutional guidelines.

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The recombinant influenza virus A/WSN/33/OVA$_{I/II}$ (WSN-OVA$_{I/II}$; H1N1) was previously generated by plasmid-driven reverse genetics [20]. The viruses used for the *in vivo* experiments were the A/Puerto Rico/8/34 (PR8; H1N1) virus and the X31 virus (A/Hong Kong/1/1968 hemagglutinin (HA) and neuraminidase with remaining six gene segments from PR8; H3N2). Heat inactivation of small volumes of virus suspension was performed in a water bath at 56 °C for 30 min and confirmed by the complete loss of infectivity in MDCK cells.

2.2. Antigen presentation assay in vitro

CD8+ and CD4+ T cells were isolated from the spleens and lymph nodes of OT-I and OT-II mice, respectively, by magnetic sorting (Miltenyi Biotec). CD11c-positive DCs were isolated from the spleens of C57BL/6 mice using MACS beads, and the purity ranged from 90 to 95%. DCs (10^7 cells/ml) were pre-incubated for 30 min either without chloroquine or with different concentrations of chloroquine, followed by the addition of heat-inactivated (HI) WSN-OVA$_{I/II}$ virus (1000 HAU/ml) or 1 μg/ml of OVA$_{257-264}$ or OVA$_{323-339}$ peptides, at 37 °C for 2 h. Antigen-loaded DCs were washed and incubated overnight; they were then cultured with 10^5 enriched OT-I or OT-II cells in the continued presence or absence of chloroquine. The cells were incubated for either 48 or 96 h, and 1 μCi/well [^{3}H]thymidine was added 8 h before harvesting.

2.3. Immunization of mice and challenge experiments

C57BL/6 mice were injected intraperitoneally (i.p.) with 500 HAU of the heat-inactivated X31 (HI-X31) virus (approximately

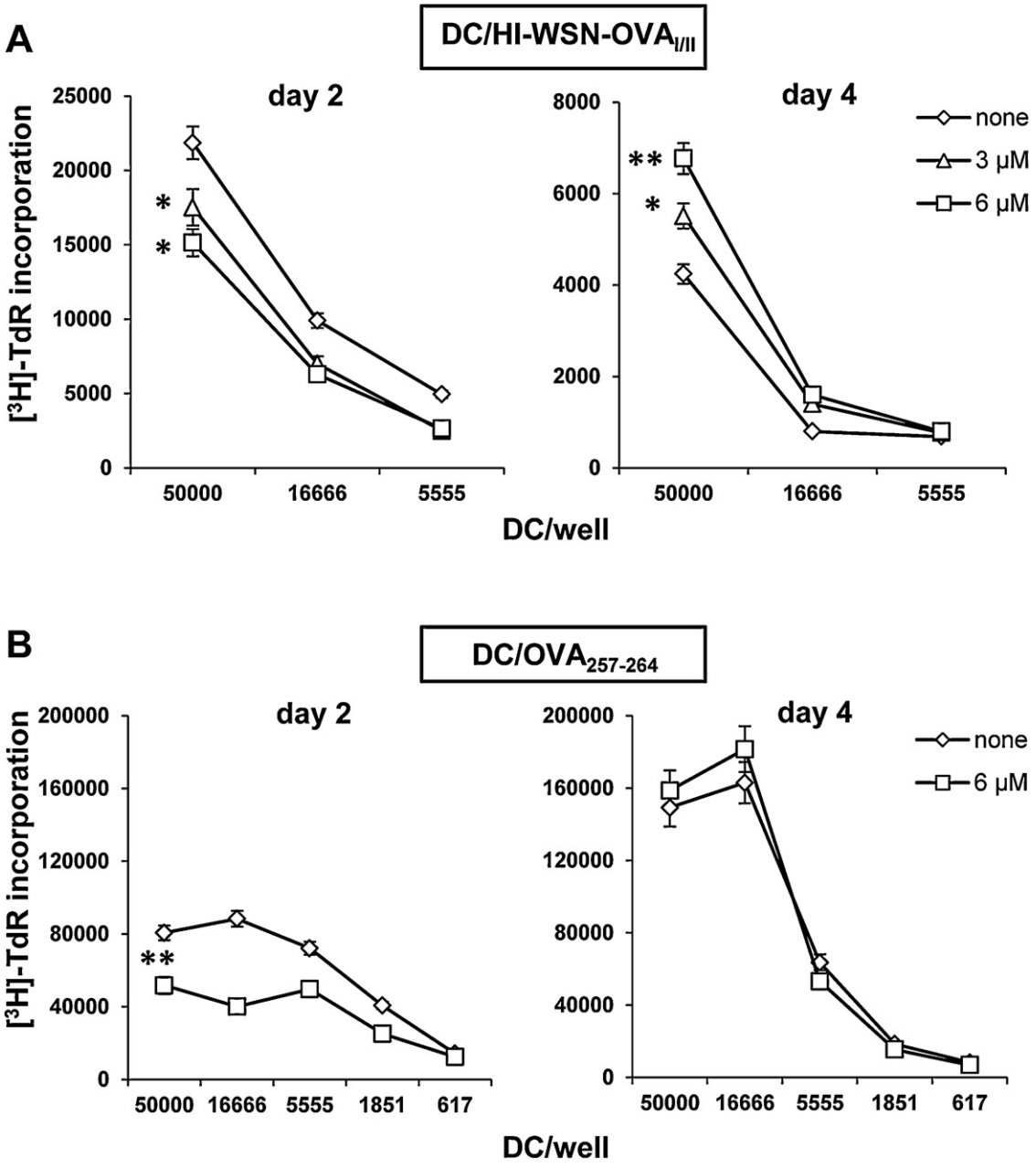

Fig. 1. Effect of chloroquine on cross-presentation by MHC class I molecules. Heat-inactivated (HI) WSN-OVA$_{I/II}$-loaded DCs (A) or OVA$_{257-264}$ peptide-loaded DCs (B) were incubated with OT-I cells in the presence or absence of different concentrations of chloroquine. T cell proliferation was estimated by use of [^{3}H]thymidine incorporation, two days and four days later. The data shown are the means (±SD) from triplicate cultures, and they are representative of three experiments with similar results. $^*P \leq 0.01$, $^{**}P < 0.001$ comparing untreated *vs.* 3 μM and 6 μM chloroquine-treated cells.

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