Data on the uptake of reducible antigen-adjuvant conjugates by dendritic cells

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This article contains the uptake data of two reducible antigen-adjuvant conjugates with different sensitivities to the extracellular and intracellular reductive environment. Using a linker with different redox sensitivity the adjuvant cytosine-phosphate-guanine (CpG) was conjugated to the fluorescently labeled model tumor antigen ovalbumin (OVA). The uptake of the conjugates by dendritic cells in a total splenocyte culture was determined using flow cytometry. The data presented in this paper supports the finding in the research article “Intracellular cleavable CpG oligodeoxynucleotide – antigen conjugate enhances anti-tumour immunity” (Kramer et al., 2016) [1].

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1. Data

This data article refers to the research article “Intracellular cleavable CpG oligodeoxynucleotide – antigen conjugate enhances anti-tumour immunity” [1]. Conjugates of the vaccine adjuvant CpG and the model tumor antigen OVA were made as improved immunotherapeutic agents. Two conjugates were synthesised, an intracellular cleavable one (HYN-SS) and an extracellular cleavable one (SS). The data here presents uptake of the CpG-OVA conjugates by a dendritic cell population of murine splenocytes in vitro. To identify fluorescently labeled conjugate taken up by dendritic cells, splenocytes
were gated on size and granularity, single cells, viability and CD11c+ dendritic cells (Fig. 1a). The intracellular cleavable HYN-SS conjugate showed statistically higher uptake compared to the extra-cellular cleaved SS conjugate (Fig. 1b).

2. Experimental design, materials and methods

2.1. Synthesis of fluorescent conjugates

OVA (Sigma–Aldrich, Saint Louis, MO, USA) was reconstituted in PBS and purified from aggregates via size-exclusion chromatography (Superdex™ 200 10/300 GL; GE Healthcare Bio-Sciences, Uppsala, SE) using PBS as the elution buffer. Purified OVA monomer was added to lyophilised NHS-DyLight633 and incubated for 1 h at room temperature. The degree of DyLight633 labelling on OVA was calculated using the extinction coefficient of 170,000 M⁻¹cm⁻¹ for DyLight633.

Oligonucleotide CpG 1668 (5’-TCCATGACGTTCCTGATGCT-3’) with a phosphorothioate backbone modified with a 3’ amine (CpG-NH₂) or 3’ thiol modification (CpG-SH) was obtained from GeneWorks Pty Ltd (Hindmarsh, SA, AUS).

For the HYN-SS conjugate, OVA-DyLight was modified with a 30-fold molar excess of succinimidyl 6-hydrazinonicotinate acetone hydrazone (HyNic from Solulink Inc., San Diego, CA, USA) in 0.1 M sodium phosphate, 0.15 M NaCl, pH 8 for 2 h at room temperature, and excess linker was removed by buffer exchange with PBS using a Vivaspin 2 spin filter. CpG-NH₂ was modified with a 30-fold molar excess of succinimidyl–SS–4-formylbenzoate (4FB-SS from Solulink) for 2 h at room temperature. HyNic-modified OVA (DyLight labeled) was reacted with the modified CpG at a 1:4 M ratio for 2 h at room temperature to form a stable bis-arylhydrazone bond. The conjugates were purified by size-exclusion chromatography (Superdex™ 200 10/300 GL).

Protein concentration of the conjugate was measured by Quant-iT™ Protein Assay Kit (ThermoFisher Scientific).

For SS conjugate, succinimidyl 6-(3-[2-pyridyldithio]-propionamido) hexanoate (LC-SPDP, Thermo Fisher Scientific) linker was prepared by dissolving it at a concentration of 20 mM in dimethyl sulfoxide (DMSO). OVA (DyLight labeled) in PBS was added to the LC-SPDP linker at a 30-fold molar excess of the linker and incubated for 30 min at room temperature. CpG-SH was added to pyridyldithiol-activated...
OVA at a molar ratio of 4:1 overnight at room temperature. The SS conjugate was purified by size-exclusion chromatography (Superdex<sup>™</sup> 200 10/300 GL) and protein concentration was measured by Quant-iT™ Protein Assay Kit (ThermoFisher Scientific).

2.2. Experimental mice

Female C57BL/6 mice aged 6–12 weeks were obtained from the Hercus Taieri Research Unit, University of Otago. Experiments were conducted in accordance with ethical permits granted by the University of Otago Animal Ethics Committee (AEC 09/14). All animals were euthanized by cervical dislocation.

2.3. Immunofluorescent analysis of conjugate internalisation

Splenocytes from C57BL/6 mice were prepared as a single cell suspension and treated with ammonium chloride to lyse red blood cells. Cells were resuspended at 1 x 10<sup>6</sup> cells/ml in cIMDM+5% FCS, plated with 100 μl per well and either pre-cooled to 4 °C or pre-warmed to 37 °C for 30 min. The cells were pulsed with 7 μg/ml of DyLight633 labeled conjugate (HYN-SS or SS). Following incubation at 37 °C, 5% CO2 or 4 °C for 1, 4 or 24 h, the cells were harvested and stained with Live Dead near IR, treated with Fc block and stained with antibodies to identify DCs (APC anti-CD11c). Fluorescence was
measured using a Gallios flow cytometer and analysed using Kaluza software version. Statistical analysis of uptake was performed using GraphPad Prism version 6.0b. Statistical analysis was carried out using one-way analysis of variance (ANOVA) with Dunnett’s post-hoc test to compare the difference of one variation in more than two different treatment groups, two-way ANOVA with Bonferroni’s post-hoc test to compare the difference of two variations in different treatment groups. The particular type of statistical analysis is indicated in each relevant figure legend. Error bars in the graphs represent standard error of the mean (SEM).

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Transparency document

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References

[1] K. Kramer, N.J. Shields, V. Poppe, S.L. Young, G.F. Walker, Intracellular cleavable CpG oligodeoxynucleotide-antigen conjugate enhances anti-tumor immunity, Mol. Ther. 25 (1) (2017 Jan) 62–70.