Stimulation of TLR7 with Gardiquimod Enhances Protection and Activation of Immune Cells from γ–Irradiation Exposure

Young-Mi Yang, Ji-Young Bang, Suhl-Hyeong Lee, Tae-Min Moon, Yu-Jin Jung
Department of Biology, Division of Life Sciences, Kangwon National University, Chunchon, 200-701, Rep. of Korea
yjjung@kangwon.ac.kr

1. Introduction

Radiotherapy for cancer patients is based on the radiation-induced cell death, but high dose of radiation is able to cause break of immune system. Thus, protection of immune cells from radiation damage is required to enhance the efficiency and reduce the harmful side effects during cancer radiotherapy.

Toll-like receptors (TLRs) are important not only in initiating innate immunity against microbial infection, but also inducing Th1-mediated immunity with producing cytokines and chemokines [1]. Cell stimulation via TLRs leads to downstream activation of NF-kB and other transcription factors. Consequently, several genes encoding mediators and effector molecules of the innate as well as the adaptive immune response are transcribed [4].

There are several previous findings that activated immune cells via TLR9 inducing pathways are resistant to chemical or radiation exposure [2,3]. But it is not clear that the other TLRs also have the same abilities to protect immune cells against cellular damages including γ-irradiation.

This research was performed to evaluate protective effect of immune cells from γ-irradiation through TLR-7 activation pathway

2. Methods and Results

2.1 Detection of TLR7 expression in Raw 264.7 mouse macrophage cell lines

TLR7 expression was detected by RT-PCR method. Total RNA was extracted under standard method using RNeasy mini kit (Qiagen, Balencia, CA, USA) and performed reverse transcription reaction and then subjected in PCR with TLR7 specific primer set.

Synthetic compounds of TLR7 ligands, Gardiquimod and Imiquimod, were purchased from Invivogen (San Diego, CA, USA) and dissolved in endotoxin free water with the concentration of 1 mg/ml and 5 mg/ml, respectively.

2.2 Enhancement of cell growth by Gardiquimod

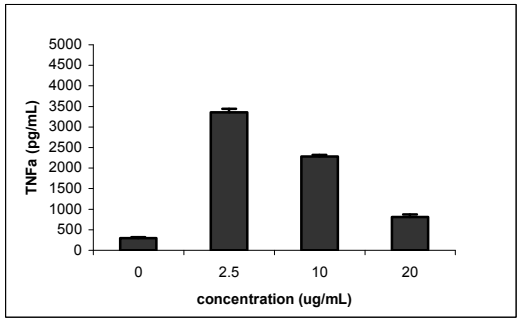
Gardiquimod induced cell growth rate more than Immiquimod, when they were treated in the growth media of mouse macrophage cell line, Raw 264.7.

2.3 Dose-dependant induction of TNFα secretion on the treatment of Gardiquimod

After treatment of TLR7 ligands, secretion of TNFα was measured as an evidence of activated immunity via TLR7 pathway. Both Imiquimod and Gardiquimod induced TNFα secretion in Raw 264.7 and mouse splenocytes. The secretion of TNFα showed dose-dependant manner in Garudiquimod treated cells, whereas Immiquimod treatment made cells secret TNFα on the peak response in 2.5 μg/ml and then decrease the level of secreting TNFα in higher doses.

Raw 264.7 cells were maintained in RPMI 1640 media containing 10% FCS. Mouse splenocytes were isolated from 8-12 weeks old C57BL/6 mouse. Cells incubated with or without TLR7 ligands for 24 hours and measured TNFα secretion using murine TNFα ELISA development kit (PeproTech, Rocky Hill, NJ, USA) according to the manufacture's instructions.

a)



b)

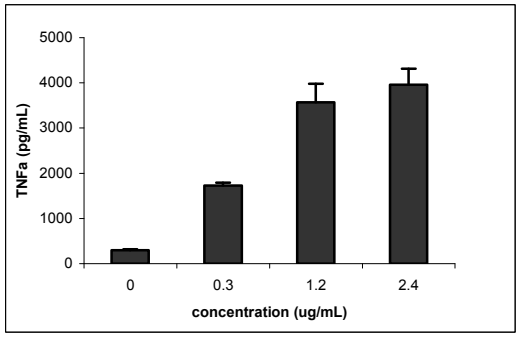


Figure 1. Effect of TLR7 ligands on the induction of TNFα secretion in mouse spleenocytes. (a) TNFα secretion showed dose-dependent declining with Imiquimode. (b) TNFα secretion was peaked on the treatment of 2.4 μg/ml Gardiquimod. The data are from two independent experiments performed in triplicate with similar results.

2.4 TLR7 ligands induced mouse splenocytes to produce IFNγ

TLR7-mediated Th1 immune response was measured with IFNγ-producing cells using murine IFNγ ELISPOT kit (BD Biosciences, San Jose, CA, USA). Mouse splenocytes were incubated in the 96 well plates which were precoated with anti-IFNγ for 24 hrs in the presence or absence of TLR7 ligands. The number of IFNγ-producing cells was detected according to the manufacture's instruction.

Gardiquimod induced more Th1 cells to produce IFNγ rather than Imiquimod not only in low but also high dose. Imiquimod was lack of ability to induce Th1 cells to produce IFNγ compared to that of Gardiquimod.

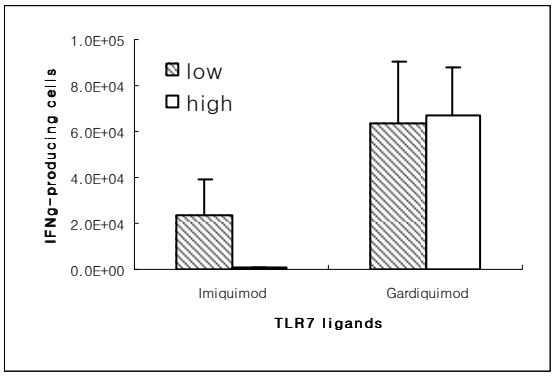


Figure 2. Stimulating effect of TLR7 ligands on the mouse splenocytes to produce IFNγ. Data are presented as mean of two experiments in triplicate.

2.5 Protection of mouse splenocytes from γ-irradiation-induced cell death by Gardiquimod

To detect if TLR7-mediated cell activation protect immune cells from γ-irradiation-induced cell death, mouse splenocytes were stained with propidium iodide (PI) and FITC or APC-conjugated cell surface marker antibodies and analyzed them in the flow cytometry. Before analyze, splenocytes were incubated with or without Gardiquimod for 6 hours and exposed low or high dose of γ-irradiation. After incubating for another 12 hours, cells were stained and subjected to FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA). Data analyze was performed using Flowjo program (Tree Star Inc., Ashland, OR, USA).

When cells were exposed under low dose (2 Gy) of γ-irradiation, more than 80% of immune cells were died in control group, whereas 2.4 μg/ml Gardiqumimod treatment made splenocytes be protected more than control group. The viability of all cell subpopulations in Gardiquimod treated group was higher more than 50% compared to that of control group.

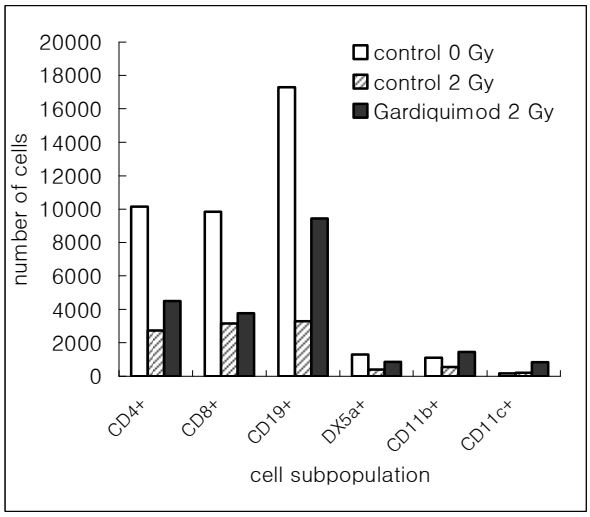


Figure 3. Protection of immune cells from γ–irradiation with pretreatment of Gardiquimod. Data are representative of three independent experiments with similar results.

3. Conclusion

TLR7 ligand, Gardiquimod, was able to enhance cell growth and secretion of cytokines, TNFα and IFNγ, which was the evidence that immunity was activated via TRL7 pathway. Gardiquimod protects immune cells from γ-irradiated cell death, also. These results showed that the potential application of synthetic TLR7 ligands in radiotherapy as an enhancer and protector for the immune cells in the exposure of γ-irradiation.

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