TLR7 Agonist Imiquimod is a Potent Vaccine Adjuvant

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ABSTRACT

We evaluated the adjuvant effects of the TLR7 agonist imiquimod as compared with GM-CSF, which has been widely used as a vaccine adjuvant. Although both topical imiquimod and intradermal GM-CSF as adjuvant effectively induced the accumulation and activation of dentritic cells (DC) in draining lymph nodes (DLN), imiquimod preferentially induced the trafficking of dermal DC to the DLN. Both GM-CSF and imiquimod augmented ova specific-CD4 T cell response in ova-tg mice when used in conjunction with CD4 peptide-based vaccines, as demonstrated by in vivo proliferation of CFSE labeled DO11.10 cells. Although both GM-CSF and imiquimod stimulated OVA specific CD8 T cell responses in OVA-tg mice when immunized with a CD8 peptide, the response induced by imiquimod was of greater magnitude. Finally, imiquimod was markedly more effective in stimulating ova specific T cell immunity with a protein-based vaccine as compared to GM-CSF. Thus, imiquimod is a potent vaccine adjuvant in promoting T cell specific responses and has the potential for clinical use as a vaccine adjuvant.

METHODS

The effect of imiquimod and GM-CSF as adjuvant on activation of DC in mouse DLN was studied using C57BL/6 mice. Mice were vaccinated with 10ug ovalbumin. Intradermal GM-CSF (5ug/mice) or topical imiquimod was applied to vaccination site on three consecutive days. The DLN cells were harvested and stained with CD11c-PE-Cy5, CD86-FITC, CD205-PE and CD8 PE-Cy7, and analyzed with FC500 flow cytometer using CXP cell quest software (Beckman Coulter).

The adjuvant effect of imiquimod and GM-CSF on peptide-based vaccines was studied using ova-tg mice DO11.10 and OT-1. Splenocytes ($10x10^6$) from DO11.10 mice or OT-1 mice were labeled with CFSE and adoptively transferred to balb/c or C57BL/6 mice respectively after peptide vaccinations. Three days later, the splenocytes from receipt mice were harvested and stained with CD4-PE and CD8 PE-Cy5, and analyzed with FC500 Flow Cytometry.

The adjuvant effect on ova protein-based vaccine was also studied using C57BL/6 mice. The mice were vaccinated with 100ug ova protein twice 10 days apart. Splenocytes were harvested. A 3 days standard IFNγ ELISPOT assay and cytokine flow cytometry were used to evaluate the immunity against OVA protein (10ug/ml).

INTRODUCTION

- Potent adjuvants are key to successful vaccination against weakly immunogenic antigens such as those involved in cancer
- DC are critical in the initiation of potent immune responses
- Both dermal DC as well as Langerhans cells have been shown to be important in initiating immune responses
- We evaluated whether topical imiquimod can recruit and activate DC populations in the skin and subsequently enhance immune responses generated during active immunization as compared to another common "DC activating" adjuvant, GM-CSF

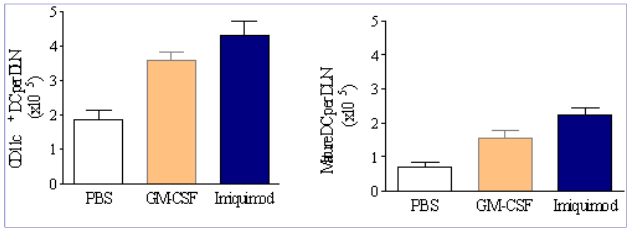

Figure 1. Both imiquimod and GM-CSF induce the accumulation and activation of $CD11c^+CD86^+$ DC in DLN. The total number of $CD11c^+$ cells per DLN and the total number of mature DC ($CD11c^+$ $CD86^{high}$) per DLN were increased 3 days after the stimulation (mature DC: GM-CSF vs. PBS, p=0.002; imiquimod vs, PBS, p=0.000; imiquimod vs. GM-CSF, p=0.052; n=5).

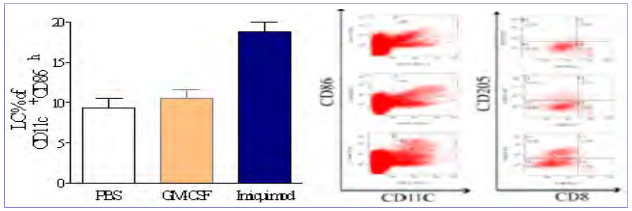

Figure 2. Imiquimod application is associated with the trafficking of dermal DC to the DLN. Percentage of Langerhans cells ($CD205^+$ $CD8^-$) in gated mature DC ($CD11c^+$ $CD86^+$) was increased in DLN of imiquimod -treated mice (imiquimod vs GM-CSF, p=0.001, n=5).

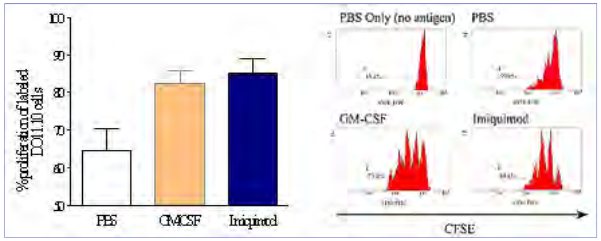

Figure 3. Both imiquimod and GM-CSF stimulate in vivo proliferation of CD4 peptide specific T cells. Balb/c mice were vaccinated with an I-Ad restricted OVA peptide (323). The proliferation of adoptively transferred CFSE-labeled DO11.10 cells was increased in vivo after the treatment (imiquimod vs. GM-CSF, p=0.428; n=4).

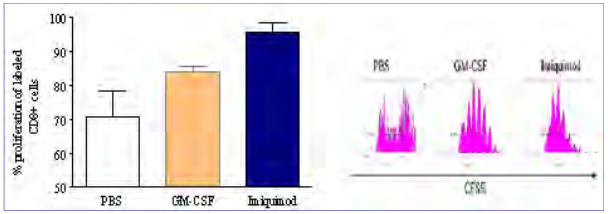

Figure 4. Imiquimod is superior to GM-CSF in inducing in vivo proliferation of CD8 peptide specific T cells. C57BL/6 mice were vaccinated with a H2-Kb restricted OVA peptide (257). The proliferation of adoptively transferred CFSE-labeled OT-1 cells was increased in vivo after the treatment (imiquimod vs. GM-CSF, p=0.013; n=4).

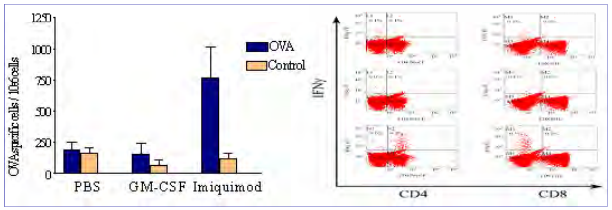

Figure 5. Imiquimod is more effective adjuvant in inducing T cell immunity with protein-based vaccine as compared to GM-CSF. Imiquimod treatment augmented ova protein-induced IFNγ secretion as detected with ELISSPOT assay (imiquimod vs. GM-CSF, p=0.039; n=6) and cytokine flow cytometry (Top, PBS; Middle, GM-CSF; Bottom, imiquimod).

CONCLUSIONS

- Imiquimod can induce maturation and migration of skin APC to draining lymph nodes
- As compared to GM-CSF, imiquimod enhances the trafficking of dermal DC which have been shown to be superior to Langerhans cell in inducing immune responses [Fukunaga A et al, J Immunol, 2008]
- Both imiquimod and GM-CSF are effective adjuvant for peptide based vaccines
- Only imiquimod could stimulate a Th1 antigen specific T cell responses when used with a protein based vaccine
- Imiquimod is a potent immunologic adjuvant for generating Th1 responses and is potentially useful for both subcomponent and protein based vaccines