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ROG 在輔助 T 細胞分化及免疫所扮演的角色

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計畫主持人：繆希椿

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ROG 在輔助 T 細胞分化及免疫所扮演的角色

關鍵詞: GATA-3，ROG，NF-AT 及 輔助 T 細胞

中文摘要

初生的 CD4 輔助型 T 先質細胞在接觸到抗原後，會分化為成熟的 Th1 或 Th2 作用細胞。Th1 免疫反應在對抗細胞內病原的防禦上很重要，並產生器官特殊性的自發性免疫反應。而 Th2 免疫反應則在對抗蠕蟲與發炎反應上扮演決定性的角色。

GATA 抑制蛋白(ROG)是個轉錄抑制因子,也是 NF-AT 的直接下游基因，可能扮演 T 細胞活化的負調控角色。GATA-3 是 T 細胞專屬的轉錄因子，為 Th2 細胞發育所必須。表現過多的 ROG 會抑制 GATA-3 的活性。因比 ROG 可能會影響輔助 T 細胞的分化及其功能。ROG 主導由 NF-AT 引發的 T 細胞活化的負回饋機制。ROG 的過度表現減低了活體 T 細胞受體(TCR)的訊息傳導。而在缺乏 ROG 的 T 細胞對於 CD3 的抗體會過度敏銳，也會增加 NF-kB 的活性，進而分泌高量的介白質素二。然而，在實驗小鼠的模式中，失去 ROG 的輔助 T 細胞仍然可以分化成 Th1 或 Th2 作用細胞，也可以產生適度的免疫反應。

在這個研究計畫中，我們進一步研究 ROG 在以下兩個領域的功能：(1) ROG 的交互作用蛋白在免疫系統中的角色；(2) ROG 基因的轉錄調控。對於第(1)項的研究，我們利用酵母菌雙雜交的模式，在脾臟細胞中篩選出 ROG 的交互作用蛋白。正在進一步研究那些 ROG 的交互作用蛋白會協助 ROG 來調節輔助型 T 細胞的活化反應。對於第(2)項的研究，我們採用細胞激素及免疫抗體的混合方法，發現 ROG 在初生的 T 細胞的基因表現是受介白質素四的抑制。而且這樣的抑制作用是藉由一個重要的訊息傳導分子,STAT6,來達成。

The Role of ROG in T Helper Cell Differentiation and Immunity

Keywords: GATA-3, ROG, NF-AT, and T helper cells

ABSTRACT

Upon encountering antigen, naïve $CD4^+$ precursor helper T (Th) cells differentiate into mature effector Th1 and Th2 cells. Th1 responses are important in defense of intracellular pathogens and responsible for mounting organ–specific autoreactive immune responses. Th2 responses are known to be critical in anti-helminthic and anti-inflammatory reactions.

Repressor of GATA (ROG), a transcriptional repressor, is a direct target gene of NF-AT and a putative negative regulator of T cell activation. GATA-3, a T cell-specific transcription factor, is essential for the development of the Th2 cell lineage. In addition, overexpression of ROG suppressed the activity of GATA-3, suggesting a role of ROG in the differentiation and function of Th cells. ROG is mediated a negative feedback mechanism of T cell activation by NF-AT (Nuclear Factor of Activated T cells). Overexpression of ROG attenuates the TCR signaling *in vivo*. ROG-deficient T cells are hypersensitive to anti-CD3 stimulation and produce more IL-2 due to enhanced NF-kB activity. However, ROG-deficient Th cells are capable of differentiating into Th1 and Th2 cells and ROG-deficient mice have no defect in mounting appropriate Th immune responses *in vivo*.

In this research plan, I further studied the function of ROG in two related areas:(1) the role of ROG-interacting proteins in the immune system, and (2) the transcriptional regulation of ROG. In conclusion, we have cloned ROG-interacting partners from spleen cDNA library by using the yeast-two-hybrid system. We are analyzing the function of them in the immune response aggressively. Furthermore, the cytokines IL4, but not interferon-gamma (IFNγ), has the role in controlling the expression of ROG in the naïve $CD4^+$ T cells. The IL4 inhibits the expression of ROG transcripts through STAT6 signaling.

Introduction

Upon encountering antigen, naïve $CD4^+$ precursor helper T (Th) cells differentiate into mature effector Th1 and Th2 cells. Th1 responses are important in defense of intracellular pathogens and responsible for mounting organ–specific autoreactive immune responses. Th2 responses are known to be critical in anti-helminthic and anti-inflammatory reactions. GATA-3 is a cell-specific transcription factor and is essential for the development of the Th2 cell lineage. ROG, a new member of the POZ family of transcriptional repressors, was initially cloned as a GATA-3 interacting protein. Resting T cells express ROG at a very low level, but is rapidly induced upon stimulation. In vitro, ROG can serve as a GATA-3-dependent transcriptional repressor, but unlike GATA-3, ROG transcripts can be detected in both Th1 and Th2 cells. Furthermore, over-expression of ROG in Th clones inhibits cytokine production. Recently, we discovered that ROG is mediated a negative feedback mechanism of T cell activation by NF-AT (Nuclear Factor of Activated T cells).These studies suggest that ROG might play a role in T cell activation in addition to serving as a GATA-3-dependent transcriptional repressor.

I took one step further to study the function of ROG in two related areas:(1) the role of ROG-interacting proteins in the immune system, and (2) the transcriptional regulation of ROG. We are aimed at understanding the mechanisms regulating the T helper cell differentiation and immunity by the transcription repressor ROG and ROG-interacting protein.

Specific aim 1. To identify the interacting proteins of ROG.

ROG was first cloned with the yeast two-hybrid system by using Zinc finger domains of GATA as a bait and an activated-Th2 cDNA library (Miaw et al., 2000). GATA-3 is essential for the development of the Th2 cell lineage. The expression of GATA-3 is further induced in Th2 cells but not in Th1 cells. ROG, the POZ family of transcriptional repressors, has different expression pattern as GATA3. ROG is a lymphoid-specific gene. Resting T cells express ROG at a very low level, and is rapidly induced within 4-6 hours upon stimulation with anti-CD3. In vitro, ROG can serve as a GATA-3-dependent transcriptional repressor, but unlike GATA-3, a Th2 cell specific transcription factor, ROG transcripts can be detected in both type 1 Th and type 2 Th cells. Furthermore, over-expression of ROG inhibits both Th1 and Th2 cytokines production in Th clones (Miaw et al., 2000). Recently, a human homolog of ROG, PAZF, has been shown to be involved in the cell cycle regulation in myeloid cell lines (Dai et al., 2002). By studying the transcription regulation of ROG, we

discovered that ROG is mediated a negative feedback mechanism of T cell activation by NF-AT. We identify that ROG is a direct downstream target gene of NF-ATc2 *in vitro* and *in vivo*. The NF-ATc2 directly transactivates the promoter of ROG via a functionally critical NF-AT site. The induction of ROG is impaired in Th cells obtained from NF-ATc2-deficient mice. Furthermore, partial restoration of ROG expression attenuated the hyperproliferation of NF-ATc2-deficient or NF-ATc2/NF-ATc3 double deficient T helper lymphocytes *in vitro* and *in vivo*. The anti-proliferation effect of ROG, indicated by the Western analysis of phospho-proteins, is mediated by attenuating signals generated from T cell receptors (Miaw et al., 2004).

Taken together, these observations suggest that ROG might play a role in T cell activation in addition to serving as a GATA-3-dependent transcriptional repressor. ROG contains a BTB and a zinc finger domain, both of which have been shown to be capable of mediating protein-protein interaction. Thus, it is likely that there are other ROG interacting proteins. In order to address this possibility, I attempted to clone interacting partner(s) by using the yeast two-hybrid system.

Specific aim 2. To identify the upstream signals which regulate ROG.

The main focus of this proposal is to identify other upstream signals that regulate ROG transcription. As we know, the lineage commitment of Th cells is largely controlled by cytokines. For example, the Th1 lineage commitment is mainly dependent on interferon-gamma (IFNγ) whereas the Th2 lineage commitment is largely controlled by IL4. T-bet, a master regulator of the Th1 lineage commitment, is rapidly induced by IFNγ through STAT1 signaling (Lighvani et al., 2001; Szabo et al., 2002). Moreover, my preliminary data show that induction of ROG by anti-CD3 is preferentially in the primary Th1 cells versus in Th2. This observation suggests that the cytokines might have the role in controlling the expression of ROG in the naïve $CD4^+$ T cells. Therefore, I attempted to identify the cytokines regulating transcription of ROG.

Experimental design and methods for specific aim 1.
To identify the interacting proteins of ROG.

I cloned possible interacting partner(s) with the yeast two-hybrid system. The ‘prey’ is libraries of plasmid pACT2 expressing cDNAs from human spleen (Clontech). They have been transformed into a yeast strain, Y187.

The ‘baits’, two different domains (BTB and POZ) of ROG, have been cloned separately into a plasmid, pGBKT-7. They have been transformed into a yeast strain, PJ69-2A. Then, mated the bait and library cultures and streaked transformants that

can grow on selection media. Performed β-gal colony-lift filter assay, isolated clones from positive yeast clones, and identified and purified the prey from *E.coli* transformants. Reconfirmed the assay for the true positive clones. The clones have been grouped according insert size and AluI and/or HaeIII restriction patterns. Then, we have performed sequence analysis.

Experimental design and methods for specific aim 2.

To identify the upstream signals which regulate ROG.

The lineage commitment of Th cells is largely controlled by cytokines. My preliminary data show that induction of ROG transcripts by anti-CD3 is preferentially in the primary Th1 cells versus in Th2. This observation suggests that the cytokines might have the role in controlling the expression of ROG in the naïve $CD4^+$ T cells. We identified the regulation of ROG by upstream stimuli by treating murine naïve $CD4^+$ T cells with different set of cytokines/antibodies combinations which mimic the Th1-skewed or Th2-skewed conditions and analyzing the level of ROG expression by Northern analysis and real-time PCR.

Results

Identification the interacting proteins of ROG.

ROG contains a BTB and a zinc finger domain, both of which have been shown to be capable of mediating protein-protein interaction. Thus, it is likely that there are other ROG interacting proteins. In order to address this possibility, we have cloned interacting partner(s) by using the yeast two-hybrid system. To date, we obtained 100 positive colonies for the screening. We are analyzing the function of them in the immune response aggressively.

Identification the cytokine regulating transcription of ROG.

The cytokines IL4, but not interferon-gamma (IFNγ), has the role in controlling the expression of ROG in the naïve $CD4^+$ cells. Moreover, the IL4 inhibits the expression of ROG transcripts through STAT6 signaling (Fig.1).

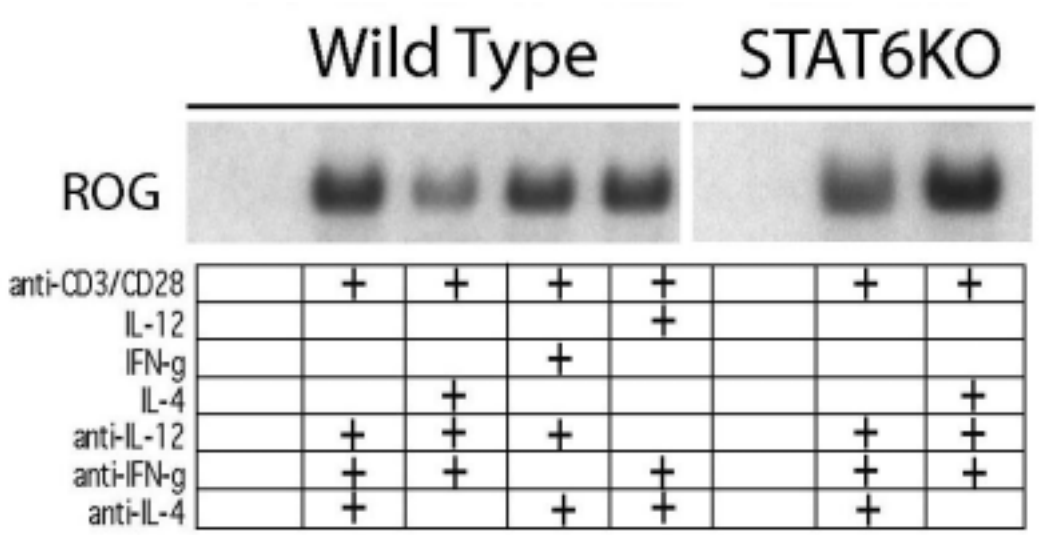

Fig.1 The expression of ROG by cytokines in the naïve $CD4^+$ T cells

Conclusion

The goal of this proposal is to study the molecular mechanisms by which ROG regulates Th2 cell differentiation and immunity. More specifically, I hope to study the transcriptional regulation of ROG, and the role of ROG-interacting proteins in the immune system. At the end of this project, we expect to have better understandings of the function of transcription repressor ROG in the immunity.

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