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ผลของ α -Galactosylceramide (แอนติเจนที่จำเพาะต่อ iNKT cells) ต่อการเปลี่ยนแปลงของไซโตไคน์ ในหนู BALB/c

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The Effect of α -Galactosylceramide, Specific Antigen for iNKT Cells, on Cytokine Profiles in BALB/c Mice

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หลักการและวัตถุประสงค์: Invariant natural killer T (iNKT) cells คือเซลล์ที่อยู่ในกลุ่มของ T cells ที่หลั่งไซโตไคน์ (cytokine) หลายชนิดเพื่อตอบสนองต่อแอนติเจน (antigen) ที่เป็นลิพิด (lipid) หรือไกลโคลิพิด (glycolipid) ที่นำเสนอบน CD1d ของ antigen presenting cells α -Galactosylceramide (α -GalCer) เป็นแอนติเจนที่จำเพาะต่อ iNKT cells โดยหลังจากการถูกกระตุ้น ภายในเวลา 1 ชั่วโมงจะมีการหลั่งทั้ง โปรอินเฟลมมาทอรีไซโตไคน์ (proinflammatory cytokine) และ เรกูลาทอรีไซโตไคน์ (regulatory cytokine) เพื่อกระตุ้นเซลล์อื่นๆในระบบภูมิคุ้มกัน ดังนั้นการศึกษานี้จึงมีวัตถุประสงค์เพื่อศึกษาการเปลี่ยนแปลงของไซโตไคน์ในหนู BALB/c ที่ถูกฉีดด้วย α -GalCer ในช่วงเวลาต่างๆ

วิธีการศึกษา: หนู BALB/c ถูกฉีดเข้าช่องท้องด้วยแอนติเจน α -GalCer ปริมาณ 1 ไมโครกรัมต่อหนูหนึ่งตัว และเก็บตัวอย่างเลือดที่เวลา 1, 2, 4, 6, 12 และ 24 ชั่วโมงภายหลังการฉีด ตัวอย่างเลือดจะถูกนำมาตรวจวัดปริมาณไซโตไคน์ด้วย Cytometric Bead Array

ผลการศึกษา: GM-CSF, IFN- γ และ IL-4 เป็นไซโตไคน์หลักที่ตรวจพบได้ในปริมาณสูงในช่วงแรกของการทดลอง โดย IFN- γ เป็นไซโตไคน์ที่มีปริมาณสูงที่สุดหลังการฉีด α -GalCer นอกจากนี้ IL-10, IL-13 และ IL-17A ตรวจพบได้ในช่วงแรกที่ปริมาณต่ำและเพิ่มสูงในช่วงหลัง อย่างไรก็ตามปริมาณที่ตรวจได้ไม่สูงเมื่อเปรียบเทียบกับกลุ่มไซโตไคน์หลัก

สรุป: ผลจากการฉีดแอนติเจน α -GalCer 1 ครั้งในหนู BAL-

Background and Objective: Invariant natural killer T (iNKT) cells are T cells subset that can secrete various cytokines respond to lipid or glycolipid antigens presented by CD1d on antigen presenting cells. α -Galactosylceramide (α -GalCer), the specific antigen for iNKT cells, can activate iNKT cells to secrete both proinflammatory and regulatory cytokines to activate the other immune cells. This study aimed to investigate the kinetic of cytokines in BALB/c mice at several time points after α -GalCer injection.

Methods: BALB/c mice were intraperitoneally injected with 1μg of α -GalCer per mouse and collected the blood samples at time points 1, 2, 4, 6, 12 and 24 hours post-injection. The samples were determined the cytokine levels by Cytometric Bead Array.

Results: GM-CSF, IFN- γ and IL-4 were the major cytokines detected in high levels in the early time point. IFN- γ was the highest cytokine levels. IL-10, IL-13 and IL-17A were low levels at the early time points and increased at the late time points. However, the levels were not high when compared to major cytokines.

Conclusions: The consequence after single dose injection of α -GalCer in BALB/c mice showed the

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B/c แสดงถึงการเปลี่ยนแปลงของไซโตไคน์และ IFN- γ คือไซโตไคน์ที่ตรวจวัดได้ในปริมาณสูงที่สุด จากผลการทดลองปริมาณของไซโตไคน์ในแต่ละช่วงเวลาทำให้การทดลองนี้จัดเป็นข้อมูลเบื้องต้นในการออกแบบการทดลองสำหรับการศึกษาเพื่อศึกษาบทบาทของ α -GalCer ในโรคต่างๆได้

คำสำคัญ: หนูสายพันธุ์ BALB/c, การเปลี่ยนแปลงของไซโตไคน์

cytokines kinetic in each time-point and IFN- γ was the highest detectable cytokine. From the cytokine kinetic studied, this experiment is an alternative model could be used as data for the α -GalCer experimental design in various diseases.

Keywords: iNKT cells, α -GalCer, BALB/c mice, cytokine kinetic, IFN- γ

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Introduction

Invariant natural killer T (iNKT) cell is a T lymphocyte subset that share properties of both T lymphocyte cells (T cells) and natural killer cells (NK cells). iNKT cells use their specialized T cell receptor (TCR) to recognize the lipid and glycolipid antigens which are presented by CD1d (MHC class I-like molecules) to become the activated cells[1, 2]. After activation, iNKT cells can secrete many cytokines and chemokines to contribute the other immune cells, for example, Th1 and Th2 cytokines and DC super-activation to prime adaptive CD4 and CD8 T cell responses. Moreover, iNKT cells can provide help directly to B cells for antibody production and rapidly activate NK cells to ready to be effector cells. From this ability, led iNKT cells to be the bridge between innate and adaptive immune responses[3].

α -Galactosylceramide (α -GalCer) is the glycolipid synthetic compound that can strongly activate iNKT cells within an hour of α -GalCer injection and after injection iNKT cell can secrete a lot of proinflammatory and regulatory cytokine[4, 5]. The GM-CSF, IFN- γ , IL-4, IL-10, IL-13 and IL-17 producing iNKT cells after α -GalCer injection are the feature of this cells[6]. GM-CSF, IFN- γ and IL-17 are the proinflammatory cytokines as well as IL-4, IL-10, and IL-13 are the regulatory cytokines[7]. α -GalCer was used in many microbial infection such as *Streptococcus pneumoniae, Pseudomonas aeruginosa, and Mycobacterium tuberculosis* to enhance bacterial clearance function of immune cells[8, 9]. In addition, α -GalCer had been used broadly in both *in vitro* and *in vivo* experiments for mouse and human iNKT cells. In this study, we represent the kinetics of cytokine after α -GalCer injection into BALB/c mice at several time points to be the data for using this compound in the other experiments in BALB/c model. Because of cytokine secreting at each time point in α -GalCer administrated mice were different, the suitable timing in this an experiment was monitored.

Materials and Methods

Animals

Six week-old male BALB/c mice were housed under specific pathogen-free conditions and maintained in the animal care unit at The University of Tokyo. Mice were purchased from SLC Japan, Inc. This study was approved by the Animal Ethics Committee of Khon Kaen University (Record No. ACUC-KKU-65/2559, Reference No. 0514.1.75/85)

α -GalCer administration

Three BALB/c mice received intraperitoneal injection with 1 μg of α -GalCer in 100 μl of PBS. Blood (100μl) was collected via retinal vein at 0 hour before α -GalCer injection, 1, 2, 4, 6, 12 and 24 hours after α -GalCer injection for determining the cytokine levels.

Cytokine measurement

Blood samples were collected from α -GalCer injected BALB/c mice at several time points. Concentrations of GM-CSF, IFN- γ , IL-4, IL-10, IL-13, and IL-17A were measured by Cytometric Bead Array (CBA; BD Biosciences), according to the manufacturer's instructions. Data were analyzed by FCAP software (BD Biosciences).

Statistical analysis.

The statistical significance of difference was determined by Student's t-test was used to compare data between time point in each cytokine. The combined data that follow a normal distribution are reported as were presented as the mean ± the standard deviation (SD). The level of significance for all statistical analyses were set at $p < 0.05$.

Results

At 1 hour after α-GalCer injection, GM-CSF was the first detectable cytokine even though it showed slightly low amount (mean ± SD, 0.500 ± 0.100 pg/ml). It was significantly increased at 4 hours (65.973 ± 4.784 pg/ml) and decreased at 6, 12 and 24 hours (43.136 ± 1.033, 37.180 ± 8.58 and 7.990 ± 0.475 pg/ml, respectively) (Figure 1A). The IFN-γ level was detected at 4 hours (48.510 ± 7.784 pg/ml) and increased the highest level at 12 hours (1054.207 ± 88.071 pg/ml) after that it was dramatically declined at 24 hours (261.773 ± 19.88 pg/ml) (Figure 1B). IL-4 was detectable the high level at 4 hours (212.1 ± 30.618 pg/ml) and started to decease at 12 hours (Figure 1C). IL-10, IL-13 and IL-17A showed the highest level at 24 hours (60.297 ± 7.784, 6.823 ± 0.263 and 18.753 ± 0.765 pg/ml, respectively) (Figure 1D, E and F).

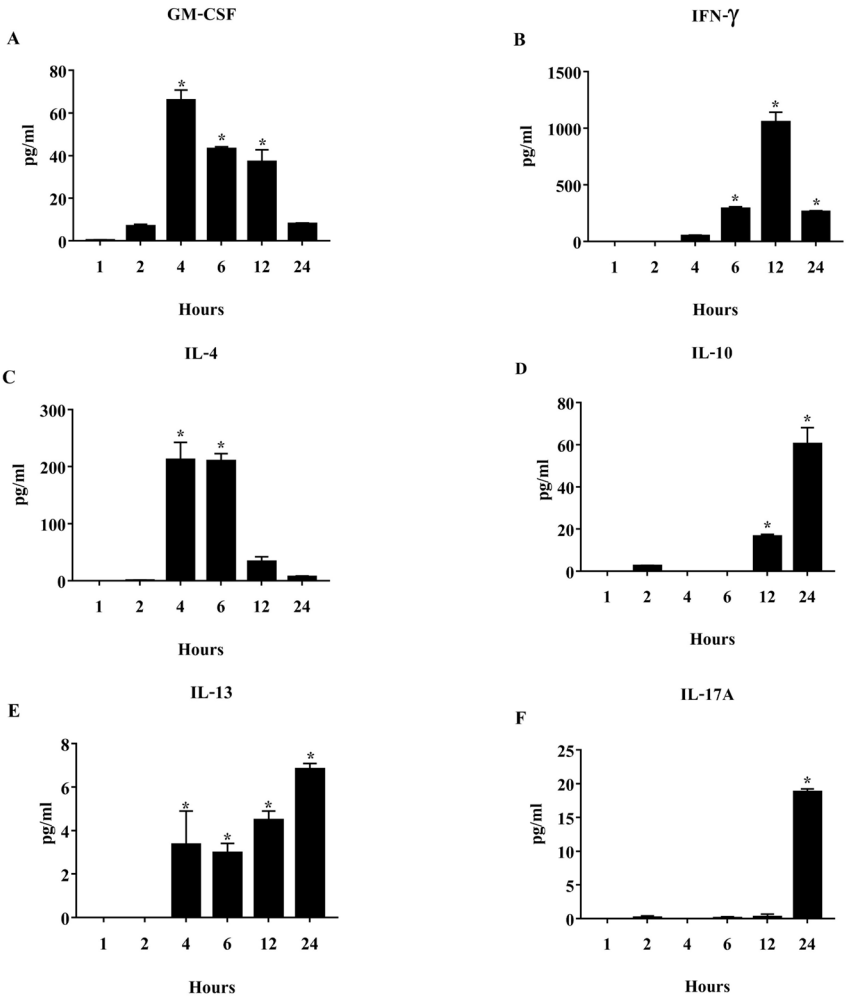

Figure 1 Kinetic of cytokine in α-GalCer injected BALB/c mice. Mice were injected with 1 μg of α-GalCer and collected the blood samples at 1, 2, 4, 6, 12 and 24 hours after injection. The serum level of GM-CSF (A), IFN-γ (B), IL-4 (C), IL-10 (D), IL-13 (E), and IL-17A (F) were detected by using BD Cytometric Bead Array. Data are mean ± SD of mice per cytokine per time point. Statistically significant differences were evaluated using Student t-test. The asterisks (*) indicate statistical significance ($p<0.05$) when compared with 1 hour after α-GalCer injection.

Discussion

iNKT cells were discovered more than 20 years. The first compound was used to identify iNKT cells was α-GalCer which is extracted component from marine sponge *Agelas mauritianus* and has been showed the antitumor activity in B16 melanoma model[2]. iNKT cells are subset of T lymphocyte that can link between innate and adaptive immune responses. Unlike conventional T cells, iNKT cells are activated by lipid or glycolipid antigens presented on antigen presenting cells (APCs). The α-GalCer is a glycolipid synthetic compound that specific for both human and mouse iNKT cell activation and strongly binding to iNKT cell TCR. The stimulation iNKT cells

with α -GalCer results in rapidly production of proinflammatory and regulatory cytokine and increase dendritic cells maturation[4]. This phenomenon is required for initiation of adaptive immune response and could be expand the antigen specific response by using α -GalCer as a co-injection[1, 3]. There are many reports present iNKT cell cytokine production by the number of intracellular cytokine staining but not cytokine response during α -GalCer injected BALB/c mice. In this study, we present the cytokine kinetic after α -GalCer injection into BALB/c mice at several time points. The cytokines in this experiment were common cytokines that released during activation of iNKT cells[1, 10]. Moreover, these cytokines had some role in some infections and diseases. Rymarchyk and colleagues reported about the genetic background between C57BL/6 and BALB/c mice after α -GalCer administration (100 ng/g body weight of mouse) and measured the cytokine levels from serum at 2, 12 and 20 hours after α -GalCer injection. TNF-α , IL-4 and IL-13 from C57BL/6 mice were significantly higher than BALB/c mice at 2 hours after administration. Whereas, IFN-γ in serum from BALB/c mice showed the significantly higher than C57BL/6 mice at 12 hours after administration. The results indicated that cytokine production resulting from NKT-cell activation is significantly different between these two inbred strains[11]. The highest IFN-γ levels in BALB/c mice could detectable at 12 hours after injection was similar time point to our result. In contrast, IL-4 was detected at 2 hours after injection but we could find a very low levels in the same time and increased in high level at 4 hours. Because 1 μg per mouse was used in our experiment which was different from those of Rymarchyk and colleagues (2.5-3 μg per mouse), such different concentration of α -GalCer might make this difference detectable cytokine levels[11]. α -GalCer was used mostly at dose 1-5μg per mouse dependent on the experiment showed that increasing cytokine levels were in dose-dependent manner[12-14]. For the routes of α -GalCer injection, many routes have been reported that the alpha-GalCer levels were depended on the agent administration technique such as intravenous, intraperitoneal or subcutaneous in α -GalCer treatment B16 melanoma studies,[15] intraperitoneal injection in α -GalCer induced liver injury study,[16] α -GalCer administration in Mycobacterium tuberculosis infected mice studies 12 and iNKT cell anergy study[17, 18]. The time point of administration, injecting α -GalCer via intravenous route immediately before tumor inoculation was not showed an anti-tumor effect[19] but pre-treatment of a single-dose α -GalCer 2 days in the same route before B16 cell inoculation leads to powerful anti-metastatic[20]. A single dose of α -GalCer (100 μg/kg body weight of mouse) via intraperitoneal injection 1-day post Mbt infection led to prolong survive than day -1, -3, -5 before infection and day 5, 9 post infection[12]. The time points of α -GalCer injection was impact on the ensuing immune response[20]. However, the cytokine levels were dependent on the dose of α -GalCer and type of mouse that used in the experiment. The point of this study was the cytokine levels in different time points and the time frequency in detection cytokine levels that was difference to the other reports[11, 18, 21, 22]. The several time points of this experiment showed the cytokines secretion in each time point was different therefore the frequency of time was option to design the experiment. Moreover, almost reports showed the intracellular cytokine producing iNKT cells more than the cytokine production consequence after α -GalCer injection in mouse[14, 18, 22]. In Mtb infection, α -GalCer administration showed prolong survival in Mtb infected mice and they found that GM-CSF produced iNKT cells after co-cultured with Mtb-macrophages play role in inhibit bacterial growth[12, 23, 24]. Moreover, α -GalCer injection during Mtb infection led to high IFN-γ production resulting in reduction of bacterial burden in the lung and spleen[12]. *Listeria monocytogenes* infected found α -GalCer injection 1 day before injection showed 100-fold reduction of bacteria in spleen and liver when compared to untreated mice. In addition, IFN-γ producing iNKT cells after α -GalCer injection promoted killing *L. monocytogenes* within phagosomes of macrophages[13]. The production of IL-4 by iNKT cells induce B cells response during the initial stage of viral infection[25, 26]. In addition, IL-4, IL-10, IL-13 and IL-17 secreting iNKT cells are play role in asthma and allergic airway disease[27-30]. In summary, after α -GalCer injected in BALB/c mice showed the cytokine kinetic and the major cytokine which detected in the early time points was GM-CSF, IL-4 and IFN-γ . In addition, IFN-γ was the highest cytokine level after α -GalCer injected. While iNKT cells activation by α -GalCer, the major cytokines were secreted to prompt the other immune cells to play role in host defense against pathogen, anti-tumor activity and some disease. However, the

time point of administration is important for experimental design suitable timing of immune response as researcher hypothesize. This cytokine kinetic can be the choice for the α -GalCer administrated in BALB/c mice experimental design.

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