

## 論文の内容の要旨

### 論文題目

The Cord Blood Levels of Heavy Metals and Trace Elements  
and Their Relationship with Immune Functions of Mononuclear Cells  
in The Babies Born in Surabaya, Indonesia

インドネシア、スラバヤにおける新生児さい帯血中の  
金属および微量元素濃度と単核球の免疫機能との関連

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### **Introduction**

Immune system is indispensable for all living organism. Allergy, infectious disease, and autoimmune would become much more threat if the immune system does not function properly. Responses of the immune system must be tightly controlled in order to prevent excessive tissue damage and the development of autoimmunity. Studies performed in the last decade clearly revealed the main role played by regulatory T (Treg) cells in maintaining immune homeostasis at the periphery.

Metals are considered as one class of the environmental toxicants, which could modulate immune function. Among whole population, individuals in early-life stages are recognized as a special subset of the population that is likely to be at greater risk to many toxicants than adults, and the toxic exposure in this stage would exert the greatest health effect in later life.

Limited human studies evaluated the effect of the heavy metals and essential trace elements on immune function of the newborn; especially, their effect on regulatory T (Treg) cells has not been examined in human. Considering such a background, this study was conducted to evaluate the effect of the element exposure during prenatal period on the immune function of newborn baby with special consideration on the two common antigens in environment (PPD and allergen/house dust mite).

Surabaya, the study place, represents cities in Indonesia, considered to be more polluted than rural area, and the participants were rice eaters. In countries where rice is consumed in large quantities, rice is considered a major source of Cd (Suzuki et al 1988) and potential contributor for Pb, which may be contained in phosphate fertilizer as impurity (Alloway, 1990).

## **Objectives**

To analyze the Cd and Pb exposure as well as Cu and Zn levels in newborn babies of Surabaya dwellers and to analyze the correlation between these elements and immune status of the newborn baby in terms of proliferation, cytokine production and subpopulation of lymphocytes.

## **Methods**

One hundred and twelve mother-infant pairs who met inclusion criteria were recruited at four health centers, representing West, East, Center and South Surabaya from March to June 2008. Informed consents were collected before sample collection. Cord

blood from at term delivery was collected after delivery prior to the expulsion of placenta by trained midwife. Cord blood mononuclear cells (CBMC) were separated within 5 hours and cryopreserved until all samples were ready for cell culture. CBMC was cultured to evaluate cell proliferation, cytokine production and T cell subpopulation. Human IL-2 was added at the beginning of the culture to induce Treg cell expansion. For proliferation assay,  $10^5$  thawed CBMC were cultured with phytohemagglutinin (Temple et al.) to stimulate mitogenesis. Proliferation of the cells was assessed at 12-14 hours (as a baseline), day 4 and 6 of the culture. Cytokine production was examined by culturing CBMC with either PHA-M, tuberculin PPD, or HDM. On day 6, IFN- $\gamma$  and IL-4 concentration in the medium were measured by Elisa. Cells harvested from the same assay were stained to evaluate subpopulation of T lymphocyte by flow cytometry; i.e., CD4<sup>+</sup>, CD4<sup>+</sup>CD25<sup>+</sup>, CD4<sup>+</sup>FoxP3<sup>+</sup>, CD8<sup>+</sup>, CD8<sup>+</sup>CD25<sup>+</sup>. Demographical as well as maternal immunological status (skin prick test for an allergen and tuberculin test) was also examined to control confounding factors for immunological outcome of baby. All statistical analyses was at  $p < 0.05$ .

This study protocol was approved by the Ethical Committee of the Graduate School of Medicine, the University of Tokyo and that of Airlangga University, Indonesia.

## **Results and Discussion**

The participating mothers aged 17 to 40 years, were with low socio-economic status, and stayed in an residential area (non-industrial area). 40.9% of them had anemia and their baby had low RBC. Interestingly, there was no sex difference in the babies in term of hematological as well as immunological parameters. Maternal factors gave strong influence

to the hematological and immunological parameters of the babies; i.e., BMI before pregnancy correlated with the number of lymphocyte, BMI at the time delivery correlated with hemoglobin, Hct, and MCV and the birth weight. Since maternal BMI had positive correlation with birth weight, which in turn correlated with CD4<sup>+</sup>FoxP3<sup>+</sup> cells, the nutritional status of the mother could influence the immune function of her baby. Parity also correlated with the number of lymphocytes. SPT of the mother correlated positively with PPD-induced IFN- $\gamma$ , and tuberculin skin test had positive correlation with HDM-induced IFN- $\gamma$  and with %CD4<sup>+</sup>CD25<sup>+</sup>.

The levels of Pb, Cd, Cu, and Zn in cord blood were similar to the reference concentration for trace elements in healthy, non-smoking adults. Cu had positive correlation with Th2 (IL-4) cytokine, and negative correlation with CD4<sup>+</sup>CD25<sup>+</sup> and CD8<sup>+</sup>CD25<sup>+</sup> cells, subpopulations of T cells with potential regulatory immune function. Zn correlated positively with the number of lymphocyte, and PPD-induced IFN- $\gamma$  production. Pb did not correlate with immune function but correlated negatively with MCH. Cd had negative correlation with MCH, and positive correlation with IL-4 production induced by PPD.

## **Conclusion**

This is the first study, which explores the prenatal exposure of metals and trace elements on immune function including Treg cells subpopulation of newborn baby.

In the 'normal level' for adult population, this study showed that Cu, Zn and Cd correlated with immune function of mononuclear cells in newborn baby.