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Activation of skin and lymph nodes antigen-presenting cells induced by *Salmonella typhi* porins

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Introduction: *Salmonella typhi* (S. *typhi*) porins are important targets of the mice and human's immune protective immune response, and are also potent immunogens capable of generating life-lasting bactericidal antibodies in mice. Mechanisms involved in this atypical antibody response remain understood. We report the activation, migration and T cell activation induction capacity of antigen-presenting cells (APC) in skin and lymph nodes in mice.

Methods: Mice were immunized intradermally with porins. Epidermis of the skin was obtained 12 h post-immunization and stained with MHC-II, CD86, CD40 and PD-L1. Tissue sections were analysed by confocal microscopy. Cervical lymph nodes were obtained and prepared for a flow cytometry staining to identify dendritic cell subsets (resident and migratory) and its activation. The capacity of porin-activated APC to activate T cell responses was evaluated by co-immunising porins with inactivated *Sporothrix schenckii* conidia. Conidia specific memory T CD4+ cells in lymph nodes were analysed by flow cytometry and in skin by a delayed-type hypersensivity test.

Results: *S. typhi* porins induced a higher expression of MHC-II and CD40 in skin, in contrast, CD86 and PD-L1 expression were not increased. Porins induced an increased number of CD86+ cells in skin despite CD40+ and PD-L1+ cells were not increased. Porins induced an increased number of migratory dendritic cells in lymph nodes which had an activated phenotype. Conidia specific total T CD4+ cells, central memory T CD4+ cells and effector memory T CD4+ cells, were increased in lymph nodes by porins co-immunization. The cellular response in skin induced by conidia-porins was higher.

Conclusion: Intradermal immunization with *S. typhi* porins induced early activation of epidermal dendritic cells and recruitment of antigen-presenting cells to skin, also promoted migration of skin dendritic cells that are able to generate memory T CD4+ cells in lymph nodes and skin, inducing systemic immune responses.

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Majority of T cells, including Treg cells, NKT and γδ T cells are developed from $CD4^-CD8^-$ T progenitor cells without the involvement of $CD4^+$ $CD8^+$ stage in thymus

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We recently have detected higher levels of Foxp3 in DN cells from mice by developing a new method of flow cytometry. In this study, we examined the expression levels of cell markers in thymocytes that exhibited an obvious change during different developmental stages of T cells. We found many cells that expressed intracellular CD4, intracellular CD8 and intracellular $CD4^+$ $CD8^+$ in $CD4^-CD8$- DN cells. The highest expression level of CD25 was observed in $CD4^-CD8^-$DN cells, followed by $CD4^+CD8^-$SP, $CD4^+CD8^+$DP and $CD4^-CD8^+$ SP cells. The expression level of CD44 in DP cells was much lower than that in the DN cells, and also recorded for $CD4^+CD8^-$ and $CD4^-CD8^+$ cells. NKT cells and γδT cells were found in DN and SP cells, but not in DP cells. The highest expression level of Notch and CD117 were observed in DN cells, followed by SP and DP cells. Unexpectedly, intracellular CD3 was not only expressed in SP and DP thymocytes, but also in most of DN thymocytes at various stages. Contaminated cells in DN thymocytes could be removed by the intracellular CD3 gated, replaced with specific blocking antibodies. Our results suggested that T cells classification has been completed in the DN thymocytes stage. T cells, including γδ T cells, NKT and Treg cells may develop from DN T progenitor cells, but without the involvement of the $CD4^+CD8^+$ stage in the thymus. We present an effective, easy and accurate method that avoids interference of contaminated cells and does not require the use of blocking antibodies to remove contaminated cells.

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