博士論文の要旨及び審査結果の要旨

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学位授与の要件	学位規則第４条第１項該当
博士論文名	Transdermal entry of yeast components elicits transient B cell-associated responses in skin-draining lymph nodes （酵母成分の経皮侵入は皮膚流入領域リンパ節における一過性のＢ細胞応答を誘発する）
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博士論文の要旨

<Background and objective> Yeasts are extensively used in vaccines, adjuvants, and drug delivery carriers/vectors. Thus, a detailed understanding of the immune responses elicited against yeasts and their components is important to effectively utilize these microbes in healthcare. Whereas, damaged or wounded skin is a popular entry route wherein skin-draining lymph nodes (sdLNs) are the sites of response. However, the type and extent of responses in sdLNs induced by non-pathogenic yeasts or their components remain to be understood. Zymosan is an insoluble component of the cell wall that comprises beta-glucans in the baker's yeast Saccharomyces cerevisiae and induces a variety of immune cells. In this study, we examined sdLN responses after the subcutaneous injection of zymosan into mice as a model to study the entry of yeast via the skin.
<Methods> C57BL/6 mice were injected subcutaneously with 0.4 mg of zymosan and sdLN (brachial, inguinal, and popliteal nodes) was collected at 8, 24, 48, and 96 hours later. The activation and proliferation of immune cells were examined by flow cytometry. Cell proliferation in lymph nodes 72 hours after zymosan administration was investigated by adoptive transferring CFSE-labeled lymphocytes. Proliferation in vitro was also tested with 72 hours culture using CFSE-labeled total lymph node cells or isolated B cells. To investigate the effects of zymosan administration on homing, lymphocytes were blocked by administrating anti-CD62L antibody. And the changes in the number of lymphocytes due to zymosan administration were compared with those of the PBS administration group. Finally, the expression of cytokine and chemokine level were compared by quantitative PCR and immunohistochemistry.
<Results> The total cell number in sdLNs increased by ~2-fold 24 h after the zymosan injection and rapidly reduced thereafter B cell number increased in response to zymosan at 24 h, whereas

T cell number was not significantly changed. Zymosan significantly induced CD69 expression in ~30% of B cells in the sdLNs after 24 h, but CD86 and MHC class-II was not significant change. Next, applicants investigated the change in B cell proliferation by zymosan in vitro and in vivo. It was detected low amounts of B cell proliferation in presence of zymosan at 72h in vitro but B cells did not proliferate in vivo. In order to clarify whether lymphocytes homing is involved in the increase of B cells in sdLNs, remained cell number ware examined by blocking the ingress of lymphocytes into sdLNs. Anti-CD62L antibody markedly reduced the total number of lymphocytes in sdLNs by ~1/3 as those in untreated samples. Even in the homing-suppressed condition, zymosan significantly augmented lymphocytes in sdLNs by primarily increasing the number of B cells. These suggest that zymosan-mediated increase in lymphocyte content in sdLNs is mainly due to the reduction of egress from the tissue. Finally, applicants analyzed the expression of cytokines and chemokines after 24 h of injecting zymosan by quantitative PCR analysis. Zymosan upregulated the inflammatory cytokines, such as IL-1beta, IL-6, IL-12, and IFN-gamma, regulatory cytokines IL-10 and TGF-beta, and lymphoid chemokine CXCL13. Among these, the expression of IL-12 and IL-10 was markedly high in B cells.

<Discussion> The inhibition of lymphocyte homing into lymph nodes did not affect zymosan-mediated increase of B cells, indicating that zymosan suppresses the egress of B cells from regional lymph nodes. We also detected specific patterns of cytokine expression in sdLNs in response to zymosan. Increased expression of IL-1B and IL-6 in the non-lymphocyte fraction indicated the induction of specific inflammatory responses. Zymosan injection also increased IL-10 and TGF expression in sdLNs. Notably, these immunoregulatory cytokines were primarily increased in the B cell fraction. We speculate that zymosan stimulates a counter response mediated by regulatory B cells.

Altogether, these findings demonstrate a unique B cell-associated response to non-pathogenic yeast component in the draining lymph nodes. This will provide insights into the clinical and healthcare applications of non-pathogenic beneficial microbes.

審査結果の要旨

発表者は、感染刺激として非病原性酵母細胞壁成分(Zymosan)を皮下注入した場合の皮膚リンパ節における免疫反応の詳細を解析し、特異的なサイトカイン群誘導反応とB cellの増殖反応を見出した。審査員からは、他の病原体を用いた刺激によるリンパ節反応に共通点があるか否か、実際の皮膚リンパ節反応は本研究結果を反映するものであるか、増殖反応を示したB cellの特異的・生物学的役割はいかなるものか、時間経過により免疫反応の推移・変化はあるか、等について質疑があった。発表者は一部の質疑に対しては応答が未熟な点もあったが、概して自己の考察を述べ適正な見解を説明することができたので、審査員一同として学位取得相当と判定した。