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学位論文名	NleC, a Type III Secretion Protease, Compromises NF-kB Activation by Targeting p65/RelA （腸内出血性大腸菌の病原因子NleCによる免疫応答抑制機構の解析）
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論文内容の要旨

〔　目　的　〕

Enterophathogenic and enterohemorrhagic *Escherichia coli* (EPEC/EHEC) are causative agents of food poisoning worldwide. Upon reaching the gut lumen, EPEC/EHEC use type III secretion system (T3SS), a needle-like protein complex, to inject effector proteins into host cells and to modify host property to enhance bacterial colonization. Various effectors have been identified yet very few are known to their functions inside the host. One aspect of the EPEC/EHEC infection is their ability to repress host immune responses thus reduces the chance of being eliminated by immune cells. However, effectors involved are not well understood. In current study, we reported the identification of a novel effector protein that can inhibit the inflammatory response and characterized the mechanism of the suppression.

〔　方法ならびに成績　〕

1. Screening of immune-modulatory T3SS-dependent effector proteins

E. coli K12 harboring functional T3SS and each individual candidate effector was used for infection of HeLa cells. Following the infection, cells were further challenged by heat-killed bacteria for an extended period of time. Degree of host inflammatory response was monitor by IL-8 or the NF-kB regulated reporter system. NleC and NleE were identified to be able to reduce IL-8 secretion and NF-kB activity in these assays. Later we decided to focus on analyzing NleC as NleE was being reported during the preparation of current manuscript.

2. Identification of host target of NleC

Since NF-kB activity was downregulated by NleC, changes in components of this pathway were examined by western blotting. RelA/p65, a subunit of NF-kBs, was found to be substantially diminished in the presence of NleC. Moreover, the reduction of RelA was not prevented in infected cells pretreated with proteosome inhibitors, indicating an alternative mean of RelA degradation directly or indirectly by NleC.

3. Functional studies of NleC

To address the possibility of direct degradation of RelA by NleC, we performed an in vitro cleavage assay mixing purified GST-NleC with HeLa lysate and analyzed the sample with RelA antibodies. We found the degradation of RelA occurred only with the presence of GST-NleC but not GST. To elucidate the mechanism of such degradation, a bioinformatic search for known domains on NleC was conducted and a zinc-protease domain was predicted. To validate this finding, mutant NleC with the defective zinc-protease domain (GST-NleCmut) was generated and tested its ability to cleave purified RelA. Results showed that the mutant NleC was unable to digest RelA. Subsequently, we further showed that a reconstituted bacteria expressing nleCmut failed to inhibit NF-kB activation in HeLa cell reporter assays. Taken together, we concluded that RelA is a substrate of NleC and the zinc-protease domain of NleC is essential for its enzymatic functions to negatively regulate NF-kB signaling.

4. Contribution of NleC in EPEC/EHEC mediated host immune-suppression

To confirm the contribution of NleC in pathogen's ability to suppress the host immune response, *nleC* knock-out mutants of EPEC and EHEC were generated. HeLa cells were infected and then challenged with the heat-killed bacteria. Degree of host inflammatory response was measured by IL-8 ELISA. Results showed that the pathogen lacking *nleC* partially alleviated the IL-8 secretion inhibition in infected cells. As NleE was also identified to be able to suppress host inflammatory response in our screening, we speculated that an additional loss of *nleE* in Δ *nleC* bacteria would result in even greater lose of immune-suppressiveness of pathogens. Indeed, we found that HeLa cells infected with Δ *nleC*Δ *nleE* secreted as much IL-8 as T3SS-defective mutant infected cells. Taken together, these results indicate that pathogens use both *nleC* and *nleE* to fully suppress host inflammatory response.

〔　総　括　〕

NleC is a RelA degrading enzyme functioning together with *nleE* for EPEC/EHEC-mediated host immune suppression.

論文審査の結果の要旨

本研究では腸内出血性大腸菌 EHEC O157:H7 の宿主の免疫応答の抑制機構に関与する因子を明らかにし、その分子機構を解明した。EHECおよびEPECの病原因子検索の新規手法を開発し、それを用いて炎症反応の抑制に関与する原因因

子として NleC 及び NleE を同定した。なかでも、NleC は NF-kB 炎症経路に必須である転写因子 p65 (RelA) を切断することによりその分解を促し、経路の活性化を阻害する事を証明した。さらにNleEとの協調的働きがEHEC/EPECの主要な炎症応答抑制機構であることを世界に先駆けて明らかにした。その重要性は EHEC O157:H7 によって引き起こす食中毒の病理現象を理解するために大きく寄与するもである。よって、この業績は学位に値するものと認める。

〔　総　　括　〕

In this study, we generated anti-NS1 MAbs cross-reacting to four DENV serotypes. These MAbs recognized three distinct regions which are conserved among all serotypes. Among them, the epitope region 2 was newly identified epitope regions. MAbs against these epitope regions, especially 2H11 and 3C4 for epitope region 2, are valuable to develop for diagnostic tools.

論文審査の結果の要旨

デングウイルス感染症は、もっとも重要な蚊媒介性のウイルス疾患であり、重症化した場合、出血傾向を伴ったショック症状から死に至る。重症化を予測する方法はなく、感染した場合、患者の状態、血液をモニターすることが重要となる。そのため感染初期での診断が重要となる。本研究では、デングウイルスタンパク質ＮＳ１が、感染初期に患者の血清中に観察されることに注目し、早期診断のためにＮＳ１を検出するマウスモノクローナル抗体を作成し、４つ存在する全てのウイルス血清型に反応する抗体を作成することができた。またこれらの抗体を用い、各血清型で交差性のある３つのＢ細胞エピトープ領域を同定した。そのうち一つは、全ての血清型に共通する新規のエピトープ領域であった。これらの研究は、早期診断法の開発に貢献すると考えられ、学位の授与に値する。