染型の invasion tip のペリプラズム内部に存在すると

Fate of 63-kDa periplasmic protein of infectious form of endonuclear symbiotic bacterium *Holospora obtusa* during infection process

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SUMMARY

Monoclonal antibodies were developed against a 63-kDa protein purified from the infectious form of *Holospora obtusa*, a macronucleus-specific endosymbiotic bacterium of the ciliate *Paramecium caudatum*. By indirect immunofluorescence microscopy, the mAbs were found to react with antigens localized in the periplasmic region of the infectious form, but not in the reproductive form of this bacterium or in other *Holospora* species. During infection, indirect immunofluorescence showed that the infectious form retained the antigens in the periplasm till they penetrated the host macronucleus. However, the antigens were no longer detected in the bacteria after invasion of the macronucleus. Instead, the fluorescence was detected in infected macronuclei, and the ratio of host cells with such fluorescence as well as the intensity of fluorescence increased with time. Whether the macronuclear fluorescence was bacterial or host in origin is unclear.

[Introduction] The gram-negative bacterium *Holospora obtusa* is a macronucleus-specific symbiont of ciliate *Paramecium caudatum*. It grows as reproductive short form (RF) (1.5-2 μm in length) by binary fission when the host cell is vegetatively growing but when the host starves, the RF stops dividing and differentiates into infectious long form (IF) (13-15 μm in length) forming a cytoplasmic region comprising half the cell length and a

large periplasmic region with a small invasion tip. At this morphologic change, the bacterium acquires its infectivity [1-3]. The accompanying increase of the periplasmic region suggests that the periplasm may contain some important molecules necessary for its infectivity. Present study aims to elucidate the function of a novel 63-kDa periplasmic protein in infection process.

[Materials and methods] *P. caudatum* strains RB-1 (syngen 4, mating type E) free and infected with *H. obtusa* strain F1 and other *H. obtusa*-bearing strains no. 18 and 24 of *P. caudatum* were used. *P. caudatum* strain no. 24 with *H. elegans* and *P. bursaria* strain Dd1g with *H. hiwatashii* were also used. Paramecia were cultivated in 1.25% (w/v) fresh lettuce juice in modified Dryl's solution[4] in which KH_2PO_4 was used instead of $NaH_2PO_4 \cdot 2H_2O$, and inoculated with a non-pathogenic strain of *Klebsiella pneumoniae* one day before use at 25 ± 1°C. IF were isolated from homogenates of *H. obtusa*-bearing paramecia by 70% Percoll density gradient centrifugation[5]. The 63-kDa protein was purified from IF by 2D-SDS-PAGE and injected into mice to develop monoclonal antibodies following routine procedures of Galfre and Milstein[6]. For infection experiments, isolated IF ($1x10^6$ ml^{-1}) were mixed with *H. obtusa*-free paramecia ($5x10^3$ ml^{-1}) and the fates of the 63-kDa protein at various time intervals after mixing were traced by indirect immunofluorescence microscopy (IIFM), suspension technique with or without treatment with 1N NaOH [7].

[Results and discussion] Ten kinds of hybridoma cells that produced monoclonal antibodies (mAbs) against 63-kDa protein of *H. obtusa* were developed. All mAbs reacted with a 63-kDa band by immunoblots. IIFM demonstrated that these antigens were localized in the periplasmic region of IF but were not were not detected in the RF or in other *Holospora* species, *H. elegans* and *H. hiwatashii.*

Only four mAbs were initially used in infection experiments, namely: mAbs 1 (2-C4-H12-G5), 3 (3-D1-B9-C4), 7 (4-G1-F4-C2) and 10 (5-C12-D9-B5). During infection, IIMF demonstrated that the antigens were kept in the periplasmic region as the IFs were engulfed in the digestive vacuoles, escaped into the host cytoplasm and penetrated the macronuclear envelop. However, the antigens disappeared from the bacteria after invading the macronucleus. Unexpectedly, however, the antigens were detected in the infected macronuclei as evidenced by macronuclear fluorescence and the ratio of such paramecia and the intensity of the fluorescence increased with time noticeably one day after infection. This phenomenon implies that the antigens may be needed after bacterial infection into the macronucleus.

The release of 63-kDa protein from the IFs during infection into the macronucleus could possibly account for the initial detection of the antigen in the infected macronucleus. However, it can not explain the successive increase of the fluorescence as time passed. Two possibilities can be considered; (1) the invading bacteria initially excreted the antigen into the macronucleus upon infection and thereafter synthesized more of the same antigen, and (2) the host possessed proteins with the same epitope as that of the IF's periplasmic 63-kDa protein and was triggered to transport the said proteins into the macronucleus in response to bacterial infection into the macronucleus. The present study therefore aims to resolve these two possibilities. It specifically aims to confirm the source of macronuclear fluorescence whether bacterial or host's, and also whether the antigens are indispensable in infection or not.

[References]

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