学 位 論 文 の 要 旨

氏 名　　蔡 杉杉

学 位 論 文 名　**Comparative Studies on the Roles of Mediator Molecules in Expression of the Suppressor Activity of *Mycobacterium avium* Complex-induced Immunosuppressive Macrophages against T Cell and B Cell Mitogenic Responses**

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著 者 名　**Shanshan Cai, Toshiaki Shimizu, Haruaki Tomioka**

論 文 内 容 の 要 旨

INTRODUCTION

Cell-mediated immunity is known to play dominant roles in protective immune responses against mycobacterial organisms including *Mycobacterium avium* complex (MAC). It has recently been reported that B lymphocytes also play roles in the early protective immune responses of hosts to mycobacterial infections. In MAC infections, generation of immunosuppressive macrophages is generally observed. These macrophage populations suppress T cell functions, especially concanavalin A (Con A)-induced mitogenesis, resulting in marked suppression of cellular immunity in the progressed stages of infection. In this study, we examined the suppressor activity of immunosuppressive macrophages induced in the spleens of MAC-infected mice (MAC-MΦs) against B cell mitogenic responses to lipopolysaccharide (B cell LPS mitogenesis). We investigated the profiles of MAC-MΦ-mediated suppression of B cell functions, with particular attention paid to the roles of suppressor mediators including reactive nitrogen intermediates (RNIs), transforming growth factor-beta (TGF-β), prostaglandin E_2 (PGE_2), free fatty acids (FFA), and phosphatidylserine (PS), in the expression of suppressor activity of MAC-MΦs.

MATERIALS AND METHODS

Microorganisms. MAC N-260 strain (*M. intracellulare* serovar 16) isolated from a patient with MAC infection was used.

Mice. Eight- to 10-week-old male BALB/c were used.

Suppressor activity of MAC-MΦs. Normal spleen cells (SPCs) were cultivated in 0.2 ml RPMI-medium containing 2 μg/ml Con A or 10 μg/ml LPS in microculture wells in the presence or absence of MAC-MΦs at 37°C for 72 h in a CO_2 incubator, and measured for ^{3}H-TdR uptake during the

final 6- to 8-h cultivation. In some cases, T cells were stimulated with immobilized anti-CD3 antibody (Ab) plus anti-CD28 Ab in order to measure T cell receptor (TCR) ligation-induced mitogenic response (net T cell mitogenesis). For assay of B cell receptor (BCR) ligation-induced mitogenic response (net B cell mitogenesis), B cells were stimulated with anti-IgM Ab plus anti-CD40 Ab. Suppressor activity of MAC-MΦs was calculated as:

% suppression of SPC mitogenesis = [^{3}H-uptake (-MAC-MΦs) - ^{3}H-uptake (+MAC-MΦs)] ÷ ^{3}H-uptake (- MAC-MΦs)×100.

Dual-chamber cultivation of MAC-MΦs and target T and B cells. Assay 1: Normal SPCs were cultivated on monolayer culture of MAC-MΦs in a 16-mm culture well (bottom chamber) in 1 ml RPMI-medium containing Con A (2 μg/ml) or LPS (10 μg/ml). Assay 2: Normal SPCs in 0.5 ml RPMI-medium were added to a 10-mm well (top chamber) equipped with a 0.45 μm Millipore filter-bottom, which was immersed in 0.5 ml medium that was poured onto an MAC-MΦ monolayer culture in the bottom chamber, and cultured in the presence of Con A or LPS. In this dual-chamber system, SPCs in the top chamber were separated from the MAC-MΦs by a Millipore membrane, which allowed free diffusion of soluble factors between the two chambers.

Measurement of RNI production. Nitrite concentrations in the culture supernatants of MAC-MΦs with or without target normal SPCs were measured by Griess assay.

Statistical analysis. Statistical analysis was performed using Bonferroni's multiple *t*-test.

RESULTS AND DISCUSSION

Effects of nitric oxide (NO) synthase inhibitor and NO scavenger on the MAC-MΦ-mediated suppression of T cell and B cell mitogenesis. In order to determine the role of RNIs (mainly NO) in the expression of suppressor activity of MAC-MΦs against T cell and B cell mitogenesis, we compared the effects of N^G-monomethyl-L-arginine (L-NMMA, NO synthase inhibitor) and 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (carboxy-PTIO, NO scavenger) on MAC-MΦ-mediated suppression of proliferative responses of SPCs to Con A and LPS stimulation. First, it was found that MAC-MΦs exhibited dose-dependent suppressor activity against both T cell Con A mitogenesis and B cell LPS mitogenesis. Second, L-NMMA as well as carboxy-PTIO specifically blocked the suppressor activity of MAC-MΦs against both T cell Con A mitogenesis and net T cell mitogenesis, while the suppressor activity of MAC-MΦs against B cell LPS mitogenesis and net B cell mitogenesis was not effectively blocked by such anti-NO agents. In addition, comparable amounts of RNIs were generated from MAC-MΦs when cocultivated with target SPCs in the presence of Con A or LPS, although RNI production by LPS-stimulated MAC-MΦs alone was much greater than that by Con A-stimulated MAC-MΦs. Therefore, unlike the major role of NO in the suppression of T cell mitogenesis, RNIs appear not to play a crucial role in the suppression of B cell mitogenesis by MAC-MΦ. It appears that certain suppressor factors other than RNIs might play central roles in the expression of the suppressor activity of MAC-MΦs against B cell mitogenesis.

Effects of scavengers for reactive oxygen intermediaters (ROIs) on MAC-MΦ-mediated suppression of T cell and B cell mitogenesis. In order to determine the roles of ROIs as mediators of MAC-MΦ-induced suppression of T cell and B cell mitogenesis, we examined the effect of the ROI scavengers glutathione and *N*-acetyl-L-cysteine (NALC) on the expression of suppressor activity of MAC-MΦs against T cell and B cell mitogenic responses to Con A and LPS stimulation, respectively. First, neither of the two ROI scavengers blocked the suppressor activity of MAC-MΦs against T cell Con A mitogenesis, indicating that ROIs do not play important roles as mediators of MAC-MΦ-mediated suppression of T cell mitogenic responses. In contrast, the same ROI scavengers markedly inhibited MAC-MΦ-mediated suppression of B cell LPS mitogenesis, implying that ROIs act as mediators in the expression of suppression by MAC-MΦs of B cell mitogenic responses. Notably, glutathione did not affect suppression by MAC-MΦs of net B cell mitogenesis induced with anti-IgM Ab/anti-CD40 Ab, presumably due to a low level generation of ROIs from MAC-MΦs without LPS-signaling. It thus appears that MAC-MΦ-derived suppressor factors other than RNIs and ROIs play important roles in MAC-MΦ-mediated suppression of net B cell mitogenesis.

Susceptibilities of T cell mitogenesis and B cell mitogenesis to the direct inhibitory effects of MAC-MΦ-derived suppressor mediators. We next compared the susceptibilities of T cell and B cell mitogenesis to the inhibitory effects of suppressor mediators, including RNIs, ROIs, FFA (arachidonic acid), TGF-β, PGE_2, and PS. It was found that T cell Con A mitogenesis and B cell LPS mitogenesis were similarly susceptible to the inhibitory effects of RNIs. On the other hand, B cell LPS mitogenesis were more susceptible to ROIs than T cell Con A mitogenesis. Notably, B cell LPS mitogenesis was less susceptible to the other suppressor mediators, including FFA, TGF-β and PGE_2, than T cell Con A mitogenesis.

Dependence of MAC-MΦ-mediated suppression of T cell and B cell mitogenesis on cell-to-cell contact with target cells. Our previous studies demonstrated that the immunosuppressive signal of MAC-MΦs was transmitted to target T cells via cell-to-cell contact. Using a dual-chamber system, we examined whether transmission of MAC-MΦ suppressor signals to target B cells also depended on cell-to-cell contact. Unlike the case of T cell mitogenesis, the suppressor activity of MAC-MΦs against B cell LPS mitogenesis was reduced only in part, when SPCs were separated from MAC-MΦ culture on the bottom chamber through a Millipore membrane. Moreover, MAC-MΦ suppressor activity was not blocked by anti-B7-1 mAb, which strongly blocks MAC-MΦ-mediated suppression of T cell mitogenesis. These findings indicate that MAC-MΦ suppression of B cell mitogenesis is largely independent of cell-to-cell contact with target B cells, unlike the case of MAC-MΦ-mediated suppression of T cell mitogenesis.

CONCLUSION

The present study demonstrated marked differences in the modes of suppression by MAC-MΦs of target T cell and B cell mitogenesis.