Down regulation of Ia expression in macrophages following incubation with mycobacteria

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key words: macrophage, IL-1, TNF, Ia, mycobacteria

Macrophages are known to release cytokines in response to various kinds of stimulators. In the present study, peritoneal macrophages from C3H/He or C3H/HeJ mice were incubated in vitro with heat-killed *M. lepraemurium*, *M. intracellulare* or *M. goodii* for 3 days followed by harvest culture supernatant to analyze cytokine activities. It, therefore, seems that macrophages phagocytizing these mycobacteria, released interleukin-1 (IL-1) and tumor necrosis factor (TNF) in culture media. The amount of release was dose dependent on mycobacteria employed. In addition, macrophages, as already have reported elsewhere, treated with IFN for 2 to 3 days showed enhanced expression of surface Ia; although the expression was inhibited if the cells phagocytized mycobacteria. Similarly, the reduced expression of Ia was observed in peritoneal macrophages from MRL/*lpr* mice after 3 day-culture with mycobacteria in vitro. More importantly, in the presence of the supernatant obtained from macrophages incubated with mycobacteria, IFN γ -treated normal macrophages exhibited suppressed expression of Ia.

These results demonstrate that cytokine release and reduced expression of surface Ia in macrophages are simultaneous phenomena after phagocytosis of mycobacteria. Suppression of Ia may be in part induced by Ia suppressive factor(s) released from mycobacterium-phagocytized macrophages.

Macrophages are defined as large mononuclear, phagocytic cells. In mycobacterial infections including leprosy, mycobacteria can invade and replicate within macrophages ⁽¹⁾.

Concerning about immunity, macrophages play an important role in immune response by production of cytokines such as interleukin 1 and tumor necrosis factor, and by presentation of fragmented-antigen to T cells through class II (Ia) molecule on their cell surface ^(2,3). Both production of cytokines and enhanced expression of Ia in macrophages are generally thought

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to be related to up-regulated immune response. We report here the production of interleukin 1 and TNF, and reduced expression of Ia in macrophage during the course of incubation with mycobacteria; these observations would be closely involved in immune response of a host against mycobacteria.

MATERIALS AND METHODS

Mice. Male or female C3H/He and C3H/HeJ mice, 2 or 3 months old were used. MRL/*lpr* mice were initially purchased from Central Institute for Experimental Animals (Kawasaki city, Kanagawa, Japan) and were maintained at our institute.

Mycobacteria. *M. lepraemurium* (Hawaii strain) were obtained from log-phase growth in nude mice. *M. intracellulare* (ATCC 15769) and *M. gordonae* (ATCC 14470) were grown in Dubos medium (Eiken, Tokyo, Japan). These mycobacteria were washed extensively with PBS, autoclaved for 5 min at 121°C, resuspended in PBS, and stored at 4°C until use.

Culture of macrophages. Peritoneal exudate cells were obtained from C3H/He or C3H/HeJ mice injected intraperitoneally with 2-3 ml of thioglycollate medium (Difco laboratories, Detroit, MICH, USA) 4 days before harvest. After harvest with Hanks buffered salt solution (HBSS, Nissui, Tokyo, Japan), 5×10^6 cells were cultured in RPMI1640 (Nissui) -5% heat-inactivated fetal bovine serum (FBS, Bio-Whittaker, Inc., Walkersville, MD, USA) supplemented with 15 mM HEPES (pH 7.2), 2 mM of L-glutamine, 100 U/ml of penicillin and 100 µg/ml of streptomycin for 2 to 3 hr in 12-well culture plates (Linbro, Flow Laboratories Inc., Mclean, VA, USA) or 60 mm dishes (Sumilon, Sumitomo Bakelite Co., Ltd., Tokyo, Japan). Non adherent cells were removed by washing with HBSS. Resulting adherent cells were then incubated for 3 days in the presence of heat-

killed mycobacteria and cell-free culture supernatant was obtained by centrifugation. After dialysis of the supernatant against RPMI1640 in a dialysis tube (Spectrapore No.3, molecular weight cut off at 3,500, Spectrum Medical Industries Inc., LA, CA, USA), filtration through 0.45 micron filter (Acrodisc, Gelman Sciences, Ann Arbor, MI, USA) was performed for analysis of IL-1 and TNF activities. Similarly, peritoneal resident cells from MRL/*lpr* mice, 3 months old in a progressing disease state, were harvested and incubated in 35 mm petri dishes for Ia analysis.

Assay for IL-1 activity. IL-1 activity in the supernatant of macrophage-cultures was determined by enhancement of thymocyte proliferation in the costimulator assay ⁽⁴⁾. One point five million of thymocytes from C3H/HeJ mice were cultured in 96-well plates (No.3596, Costar, Cambridge, MA, USA) in the presence of supernatant and 0.5 µg/ml of concanavalin A (Sigma chemical Co., St. Louise, MO, USA). Cultures incubated for a total 72 hr and pulsed with 0.5 µCi of [³H] -thymidine 18 hr before harvest cells. After collecting cells onto a glass fiber membrane, the incorporated radioactivity was counted by a liquid scintillation counter. One unit of IL-1/ml was determined as the concentration of the supernatant necessary to produce half - maximum incorporation of radioactivity.

Assay for TNF activity. The activity of TNF in supernatant was determined by a modified L929 fibroblast cell lytic assay ⁽⁵⁾. Briefly, 3×10^4 of TNF-sensitive mouse L929 fibroblast cells were cultured with diluted macrophage supernatant in the presence of 1 µg/ml of actinomycin D (Sigma) in a well of a 96-well plate for 24 hr at 37°C. After culture, medium in the plate was discarded by inversion and flicking the plate. The cells adhering to the wells were then stained with 0.1% of crystal violet and solubilized by 1% SDS solution fol-

owed by measurement of absorbance at 570 nm with a spectrophotometer (Titertek Multiscan Type, Flow Labs, Stanmore, NSW, Australia). The cytotoxic activity of TNF in units per ml was defined as the reciprocal of the dilution resulting in 50% cytotoxicity.

Ia analysis. Peritoneal resident or exudate cells were incubated in 35mm petri dishes for 2 hr, and non-adherent cells were removed by washing. Adherent cells were then cultured in the presence of IFN gamma (gift from Torei, Tokyo, Japan) for 3 days, recovered from dishes with rubber policeman and stained for Ia by using a monoclonal anti-Ia^k antibody (Becton Dickinson Immunocytometry Systems, Mountain view, CA, USA) and fluorescence conjugated F(ab')₂ fragments of goat anti-mouse IgG antibody (BioSource International Inc., Camarillo, CA, USA). Fluorescence intensity of the cells was analyzed on flow cytometry systems (FCS-1 of Japan spectroscopic Co.,Ltd., Tokyo, Japan and FACScan of Becton Dickinson Immunocytometry Systems).

LK preparation. Spleen cells were obtained from spleens of normal C3H/He mice. Briefly, spleens were obtained aseptically, minced by frost glasses, treated with 0.83% of NH₄Cl to lyse red cells, centrifuged and resuspended in serum-free RPMI1640 containing 15 mM HEPES (pH 7.2), 100U/ml of penicillin and 100 µg/ml of streptomycin in the presence of 50 mM of 2-mercaptoethanol. The cells were cultured in the presence of 2µg/ml of concanavalin A (Sigma). After 3 days, cell-free supernatant was harvested followed by mixing with Sepharose G-10 (Farmacia Chemicals, Uppsala, Sweden) and incubated for 3 hr by rotating at 4°C. The preparation was centrifuged again to remove sepharose particles and dialyzed against RPMI1640. This final preparation was used as a lymphokine-containing supernatant (LK).

Assay for glucose consumption by macro-

phages. Glucose consumption assay was performed by the methods as described previously ⁽⁶⁾. After incubation of macrophages in medium, 20 ml aliquots of the supernatant were harvested, and glucose content was assessed using a "Glucose-B-test-Wako" kit (Wako Chemicals, Osaka, Japan) which consists of glucose oxidase and so on. A macrophage activation index based on glucose utilization was calculated as: MA index = (% of glucose remaining in test sample) ÷ (% of glucose remaining in control sample).

RESULTS

Cytokine activities in supernatants harvested from macrophages incubated with mycobacteria.

Table 1. IL-1 and TNF production by mouse peritoneal exudate macrophages^a

Macrophages incubated with	IL-1 activity U/ml ^b	TNF activity U/ml ^c
<i>M.lepraemurium</i> ,		
8 µg/dish	<1	<1
40	<1	<1
200	<1	<1
1,000	7.5	13.0
<i>M.intracellulare</i> ,		
8	<1	<1
40	<1	<1
200	<1	1.8
1,000	11.6	14.8
<i>M.gordonare</i> ,		
8	<1	<1
40	<1	<1
200	<1	<1
1,000	4.8	6.7
<i>Medium</i>	<1	<1

^aMacrophages were incubated with heat-killed mycobacteria for 3 days, then IL-1 and TNF activities in the supernatants were analyzed.

^bThe activity of IL-1 was measured by a thymocyte proliferation assay.

^cThe activity of TNF was measured by a L929 cell lytic assay.

Mouse peritoneal exudate macrophages were incubated in the presence of heat-killed *M.*

lepraemurium, *M. intracellulare* or *M. gordonare* for 3 days, and cell-free culture supernatants were harvested. IL-1 and TNF activities in them were analyzed after dialysis against RPMI1640 medium.

As shown in table 1, enhanced IL-1 and TNF activities were observed in each supernatant. The results also indicated that the significant enhancement of cytokine production was observed in a dose dependent fashion of the mycobacteria employed. By acid-fast staining and observation under microscopy, macrophages incubated with mycobacteria showed many ingested bacteria inside.

Glucose consumption by macrophages following incubation with mycobacteria. Before dialysis, aliquot of the supernatant was served for measurement of glucose content by a colorimetric assay.

Table 2. Enhanced glucose consumption by mouse peritoneal exudate macrophages^a

Macrophages Incubated with	Glucose content ^b	MA index ^c
<i>M. lepraemurium</i> ,		
8 µg/dish	0.827 ± .024	0
40	0.746 ± .012	8
200	0.596 ± .007	26
1,000	0.319 ± .008	61
<i>M. intracellulare</i> ,		
8	0.766 ± .022	5
40	0.689 ± .024	15
200	0.488 ± .012	40
1,000	0.398 ± .008	51
<i>M. gordonare</i> ,		
8	0.728 ± .012	10
40	0.699 ± .024	14
200	0.485 ± .006	40
1,000	0.481 ± .009	41
<i>Medium</i>	0.810 ± .053	

^aMacrophages were incubated with heat-killed mycobacteria for 3 days, then the supernatants were collected and assessed for glucose content.

^bAbsorbance at 490nm by an enzyme assay.

^cMacrophage Activation index.

medium decreased with the increased amount of mycobacteria employed in culture, indicating the utilization of glucose by macrophages was enhanced in a dose dependent manner by the mycobacteria present in culture.

Inhibited Ia expression in macrophages which phagocytized mycobacteria. Normal peritoneal macrophages were incubated with mycobacteria and Ia expression on the cell surface was analyzed by flow cytometries. At 3 day incubation of macrophages with heat-killed mycobacteria, cells were recovered from the dishes by scraping off with rubber-policeman. The cells were then stained with mouse anti-Ia^k antibody and FITC-labelled goat anti-mouse Ig followed by analysis of fluorescence intensity. The results are shown in table 3 in the form of the mean fluorescence intensity. By drawing profiles of the results with 2nd antibody staining as a staining background, in normal cells, about 15 % population was Ia positive from a total number of cells

Table 3. Ia antigen expression in macrophages by incubation with mycobacteria^a

Macrophages incubated with	MFI (log) ^a	FI index ^b
<i>M. lepraemurium</i> ,		
8 µg/dish	27.6	0.90
40	31.7	1.03
200	22.6	0.74
1,000	14.0	0.46
<i>M. intracellulare</i> ,		
8	32.2	1.05
40	31.6	1.03
200	26.5	0.86
1,000	20.5	0.67
<i>M. gordonare</i> ,		
8	28.3	0.92
40	29.6	0.96
200	23.4	0.76
1,000	13.8	0.45
<i>Lymphokine</i>	67.2	2.19
<i>Medium</i>	30.7	

^aChannel number of MFI (Mean Fluorescence Intensity) by a flow cytometry analysis.

^bIndex of FI (Fluorescence Intensity)

= (MFI of macrophages incubated with sample) / MFI of macrophages incubated with medium).

As shown in table 2, glucose content in

(data not shown).

The logarithmic mean fluorescence intensity (MFI) of these normal cells was 30.7 (table 3). On the other hand, macrophages incubated for 3 days with three kinds of heat-killed mycobacteria, *M. lepraemurium*, *M. intracellulare*, and *M. gordonae*, all showed lower values of MFI, indicating the reduced expression of Ia. For easy understanding, the results are summarized as a fluorescence intensity (FI) index based on the consideration of fluorescence intensity of macrophages cultured in medium only as 1 (table 3).

Down-regulation of Ia expression in macrophages from MRL/*lpr* mice by mycobacteria. Since peritoneal macrophages of MRL/*lpr* mice express enhanced Ia on cell surface without any kinds of cytokine stimulations *in vitro*⁽⁷⁾, we employed macrophages of these mice as a source of Ia-positive cells. Peritoneal cells from MRL/*lpr* mice of 3 months old were harvested by intraperitoneal lavage and were seeded on dishes. After 3 hr incubation, non-adherent cells were washed out for purifying adherent cells. Over 95% of these adherent cells were macrophages as characterized by positive staining of esterase and by phagocytic activity of fluorescence-labelled latex beads. By flow cytometry analysis, the cells already possessed enhanced Ia expression on the surface (data not shown). The results in figure 1 show the suppressed Ia expression after 3 day incubation of these macrophages with heat-killed mycobacteria. Cells incubated only in medium, on the other hand, preserved high levels of Ia expression during the culture period. Peritoneal macrophages from MRL/*n*, which do not develop the disease, expressed low Ia expression during the culture period (figure 1).

Enhanced Ia expression in macrophages by lymphokine or IFN treatment and inhibited expression by incubation with mycobacteria.

Figure 1. Reduced Ia expression in macrophages from MRL/*lpr* mice following incubation with mycobacteria. Peritoneal macrophages from MRL/*lpr* or MRL/*n* mice were cultured for 3 days followed by analyzed Ia expression. In some experiments, heat-killed *M.intracellulare* were added in culture at the beginning.

Induction of Ia in macrophages by cytokine stimulation was analyzed. Peritoneal macrophages from C3H/HeJ mice were cultured with lymphokine-rich culture supernatant of spleen

Figure 2. Enhanced Ia expression in peritoneal macrophages incubated with lymphokine. Peritoneal macrophages from C3H/HeJ mice were cultured with supernatant obtained from con A-stimulated spleen cell culture. At various days after incubation, the cells were collected and analyzed for their Ia expression.

cells (LK). The results in figure 2 show that Ia expression was augmented day by day, and maximum expression was observed at 3 days. The day for analysis of Ia expression was therefore settled at 3 days. In the case of LK preparation, the Ia enhancing activity is now believed to be mainly mediated by IFN γ . Hence, IFN γ was applied to examine the induction of Ia in macrophages. The results are

Figure 3. Enhanced Ia expression in IFN γ -treated macrophages. Peritoneal macrophages from C3H/HeJ mice were cultured with several doses of murine recombinant IFN γ for 3 days. Ia expression in the cells was then analyzed by a flow cytometry. Values indicated in the lower table was calculated as a mean fluorescence intensity based on linear fluorescence intensity of each sample.

shown in figure 3. In our system, only 1 unit/ml of IFN γ was enough to induce strong Ia expression. Ten units/ml of recombinant murine IFN γ was selected for later use.

Macrophages were incubated with *M. intracellulare* in the presence of IFN γ for 3 days followed by analysis of Ia expression. As shown

Figure 4. Inability of Ia expression in mycobacterium-phagocytized macrophages. Peritoneal macrophages from C3H/HeJ mice were cultured with IFN γ in the presence of heat-killed mycobacteria for 3 days. Ia expression in macrophages were then analyzed.

in figure 4, IFN γ -treated macrophages exhibited a high level of Ia expression. Interestingly, if the cells were incubated with mycobacteria, Ia expression was strongly blocked.

Figure 5. Effect of supernatant of macrophages incubated with mycobacteria on Ia induction in IFN γ -treated macrophages. Peritoneal macrophages from C3H/HeJ mice were cultured with mycobacteria for 3 days. Then the supernatant was harvested, dialyzed, and added to normal macrophage culture with 10 U/ml of recombinant murine IFN γ . ①, 10 U/ml of IFN γ ; ②, 10 U/ml of IFN γ in the presence of supernatant obtained from macrophages incubated only with medium; ③, 10 U/ml of IFN γ and supernatant obtained from macrophages incubated with heat-killed *M. intracellulare*.

Next, macrophages were incubated concomitantly with normal or mycobacterium-containing medium for 3 days and the supernatants were harvested. After centrifugation and dialysis of the supernatants, normal macrophages were incubated with IFN γ in the presence of the supernatants. Ia expression was then analyzed after 3 days. The results in figure 5 indicate that few suppression of IFN γ -induced Ia was observed in macrophages incubated with supernatant obtained from macrophages cultured only with medium. On the contrary, strong suppressive activity existed in the supernatant of macrophages cultured with mycobacteria (figure 5).

DISCUSSION

Macrophages play a regulatory role in immune responses by production of cytokines,

interleukin-1 and tumor necrosis factor⁽²⁾, and by presentation of antigens to T cells, which recognize antigen on the surface in association with Ia molecules⁽³⁾. To explore the role of macrophages in immune response on mycobacterial infections including leprosy disease, we used three kinds of mycobacteria, such as *M. lepraemurium*, *M. avium* and *M. goodii*, and examined macrophage response to these mycobacteria in vitro. We here again demonstrated IL-1 and TNF releases by mouse macrophages following the incubation with heat-killed mycobacteria⁽⁸⁾. Furthermore we have observed TNF production by both human and mouse macrophages during the course of incubation with *M. leprae* (Fukutomi et al. in preparation). Presumably these observations are common features of macrophage response to mycobacteria. It has been reported that various mycobacterial constituents, such as cord factor, muramyl dipeptide (MDP), and LAM etc, can stimulate macrophages to produce cytokines^(9,10). Macrophage production of IL-1 and TNF following the incubation with heat-killed mycobacteria therefore would originate from response against these mycobacterial constituents.

Macrophages phagocytizing mycobacterial organisms acquired accelerated utilization of glucose, compared to normal macrophages (Table 2). Since the enhancement of glucose utilization is one of the characteristics of macrophages in the activation state⁽¹¹⁾, it is likely that mycobacterium - phagocytizing macrophages were at least metabolically activated.

Peritoneal macrophages from normal mice ordinarily express a low amount of Ia⁽¹²⁾. This was completely inhibited after the incubation with mycobacteria (table 3). In addition, similar results were obtained for macrophages of MRL/*lpr* mice. MRL/*lpr* mice develop a lupus-like syndrome characterized by antibodies to nucleic acids and by immune-complex glomer-

ulonephritis⁽⁷⁾. Peritoneal macrophages from these mice were reported to express high Ia expression preceding to IFN γ treatment in vitro⁽⁷⁾. We studied on the change in the extent of the expression of Ia on macrophages from the MRL/*lpr* mice. It was found that peritoneal macrophages from MRL/*lpr* mice incubated with heat-killed mycobacteria also indicated reduced Ia after 3 days (figure 1). IFN γ -induced increase in the Ia expression was also suppressed in the presence of mycobacteria (figure 4). Sibley et al. also reported that *M. leprae*-ingesting macrophages exhibited non-responsiveness to IFN γ , a cytokine which generally augments macrophages functions related to intracellular killing of parasites and the expression of Ia⁽¹³⁾. In any case, suppressed expression of Ia following phagocytosis of mycobacteria occurs in macrophages in any conditions.

Recently it has been pointed out LPS contamination in certain materials, specimens, and culture media in vitro study of macrophage functions, and it may affect the experimental results. LPS is a potent stimulator for macrophage cytokine producing ability but an inhibitor of IFN γ -mediated Ia induction⁽¹⁴⁾. The LPS contamination easily occurs during the preparation of specimens due to its ubiquitous existence. Therefore, there is a possibility for LPS contamination in our mycobacterial preparation. C3H/HeJ mouse, a LPS - non responder^(15,16), generally used for control experiments which are required to eliminate LPS side effects. Thus we employed macrophages from this strain of mouse as yet observed suppressed Ia following the incubation with mycobacteria, indicating that the observed suppression was not due to LPS contamination in our mycobacterial preparations.

The culture supernatant of macrophages stimulated with mycobacteria also possessed activity to inhibit LK- or IFN γ -mediated en-

hancement of Ia expression in normal macrophages. There is a report that TNF is one of the factors that suppresses Ia expression ⁽¹⁷⁾, and one or more proteins, which inhibit IFN γ -induced Ia expression, were produced early after treatment with maleylated-BSA or LPS ⁽¹⁸⁾. In addition, we observed that IFN γ -induced Ia expression was strongly inhibited by TNF (Fukutomi et al., manuscript in preparation). Cyclooxygenase product, PGE₂, which is produced by macrophages after stimulation, is also an inhibitor of IFN γ -induced Ia expression ⁽¹⁹⁾. Supernatant used in this study, which had been dialyzed to remove small molecules including PGE₂, still exhibited the suppressive activity, thereby suggesting the present suppressive factor(s) of macrophage Ia expression is not PGE₂.

Similar suppression of Ia expression in macrophages was reported by Mshana et al. for *M. kansasii* ⁽²⁰⁾. They reported that the suppression was observed only in the case of live bacteria. Live mycobacteria suppressed Ia expression without exerting no cytotoxicity. They heated mycobacteria at 100 °C for 30 to 45 min to obtain heat-killed preparation. We just heated at 121 °C for 5 min. Perhaps the difference between our and their results may be caused by the different condition of heat treatment. Namely, as they mentioned, longer treatment may alter the nature of bacterial components which are able to stimulate macrophages.

IL-1, initially described as lymphocyte activating factor (LAF), has a pivotal immunostimulant role since it can enhance both immunoglobulin production by B lymphocytes and activation of T lymphocytes ⁽²⁾. IL-1 is also known to play an important role in granuloma formation in a model system, by promoting granuloma formation ^(21,22). In addition, IL-1 activates macrophages, all functions of which activity are closely related to the protective immunity of host against bacterial infection ⁽²³⁾.

Similarly, TNF plays an important role in granuloma formation ⁽²⁴⁾. TNF displays some roles in the host resistance to bacterial infection, by activating macrophage cell functions, etc.⁽²⁵⁾. From this point of view, supernatant-induced suppression of Ia expression observed here is the other side of the coin in immune response on mycobacterial infection. Suppressed Ia expression in macrophages results in their loss of antigen presenting ability, suggesting that macrophages may lost certain kinds of cell functions without showing any decreases in their cytokine production.

As mentioned above, IL-1 and TNF mainly play as activators for macrophages rather than as inhibitors, and are probably involved in anti-*M. leprae* response in leprosy patients. In local lesion, especially in LL patients, there are many loads of bacteria in macrophages. Therefore macrophages in the lesion could produce IL-1 and TNF in response to the stimulatory signals given by bacteria as we observed in this in vitro system. It is of interest to note the findings of Yamamura et al. that there was no detectable level of IL-1 and TNF in lesions of LL patients even by using PCR technique ⁽²⁶⁾. As they mentioned in the report, immunosuppressive cytokine(s) such as IL-10 and TGF beta, which are synthesized by T lymphocytes and macrophages, may inhibit the production of IL-1 and TNF by macrophages ^(26,27,28). On the other hand, Sibley et al. reported that macrophages gorged with *M. leprae* were refractory to be activated by IFN ⁽¹³⁾. There also may be a possibility, that the macrophages phagocytized bacteria, released cytokines in a transient fashion and thereafter they lost responsiveness to any cytokine production-inducing signals by some unknown mechanisms other than cytokine-mediated suppression.

In conclusion, it is thought that macrophage responses to mycobacteria may induce changes in a variety of characteristic features of host

immune response. Moreover, cytokines released by macrophages as well as by T lymphocytes could play a crucial role in host defence against mycobacterial infections.

ACKNOWLEDGEMENTS

A part of this study was supported by a grant of US-Japan Cooperative Medical Science Program. The authors also wish gratefully to acknowledge the Sasakawa Memorial Health Foundation for the financial support for this study.

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抗酸菌と共に培養したマクロファージ上Ia分子発現の抑制調節機構

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キーワード：マクロファージ, IL-I, TNT, Ia, ミコバクテリア

マクロファージがさまざまな刺激を受けるとサイトカインを産生することが知られている。本研究では, *M. lepraemurium*, *M. intracellure*, *M. gordonare*を用いて, in vitroでC3H/He並びにC3H/HeJマウス由来腹腔マクロファージと共に培養し3日目の培養上清中のサイトカイン活性を調べた。その結果, これら抗酸菌を貪食したマクロファージからインターロイキン-1と腫瘍壊死因子(TNF)が放出されることを観察した。その放出量は加えた菌量に比例した。さらに, 以前から報告されているごとく, マクロファージをインターフェロン γ (IFN γ)と共に培養すると細胞表面上に強いIa発現が観察される。しかし,

抗酸菌を貪食したマクロファージではIFN γ によるIa発現は抑制された。同様に, MRL/lprマウスの腹腔マクロファージはIaを強く発現しているが, 抗酸菌と共に培養すると3日後には発現が抑制された。興味あることに, 抗酸菌と共に培養したマクロファージの培養上清をIFN γ 存在下で培養してもIa抑制活性が見られた。

これらの結果は, マクロファージにおけるサイトカイン産生と細胞表面Ia発現の抑制は同時に起こる現象であることを示唆している。そして, Ia発現抑制機構の少なくとも一部分は抗酸菌貪食マクロファージから産生される抑制因子によって担われていることが判明した。

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