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Profiles of the mRNA Expression by Macrophages Infected with *Mycobacterium leprae* and *Mycobacterium avium* Complex[1]

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Profiles of cytokine (CK) expression in the hosts infected with *Mycobacterium leprae* have been actively investigated. In particular, Modlin and his colleagues demonstrated that mRNA encoding Th1 CKs, including IL-2, IFN-γ, and lymphotoxin were detected at higher levels in tuberculoid lesions, but virtually absent in lesions of lepromatous leprosy patients. In contrast, Th2 CKs, such as IL-4, IL-5, and IL-10 mRNAs, were present at higher levels in lepromatous than in tuberculoid lesions ([15, 30, 33]). IL-12 produced by macrophages (Mϕs) infected with *M. leprae* facilitates a Th1 response, which is closely associated with cell-mediated immunity against *M. leprae* antigens, acts by activating NK cells to release IFN-γ, which in turn activates Th1 cells in collaboration with IL-12 ([16, 26, 30]). In the case of the host immune response against *M. leprae*, IL-12 and IL-18 produced by *M. leprae*-infected Mϕs display collaborating effects in eliciting a Th1 response ([10, 13]). Moreover, IL-7 and IL-15 produced by infected Mϕs also facilitate T-cell activation and expansion in response to *M. leprae* antigens ([11, 25]). On the other hand, IL-10 produced by Mϕs and IL-4 produced by Th2 cells, NK1.1$^+$T cells, or CD19$^+$/B220$^+$B cells suppress the Th1 response, but facilitate the Th2 response against *M. leprae* antigens ([15, 24]). Moreover, Mϕ-derived IL-10 and transforming growth factor-β (TGF-β) are known to down-regulate Mϕ antimycobacterial activity ([7, 8]). It is, thus, conceivable that Mϕs determine the host immune response against *M. leprae* infection, not only by acting as antigen processing cells or bactericidal phagocytic effector cells, but also by producing various kinds of immunoregulatory CKs.

Mycobacterium avium complex (MAC) has been reported to resemble *M. leprae* both taxonomically and biologically as follows. First, MAC and *M. leprae* are highly related and form a phylogenically tight cluster apart from other mycobacterial species, including slow growers and rapid growers ([27]). Second, although most *M. leprae* antigens have close homologs in the *Mycobacterium tuberculosis* complex ([28]), the homolog of the 35-kDa antigen of *M. leprae* is found in MAC only ([31]). Third, electron microscopic studies showed that, after the entry of MAC, as well as *M. leprae* into host cells, including Mϕs and epithelial cells, the electron transparent zone (ETZ) consisting of bacterial mycosides starts to form and surrounds these bacilli inside phagosomes ([18, 20]). The presence of an ETZ is considered to play a crucial role in bacterial resistance to the action of bactericidal effectors provided in phagolysosome vesicles ([19]). In addition, MAC organisms are frequently isolated from human lepromas. It has been reported that co-infection of MAC with *M. leprae* increases the pathogenicity of leprosy bacilli and facilitates the progression of the disease *in vivo*, presumably enhancing metabolic activity of the organisms ([12]).

These situations encouraged us to investigate profiles of the interaction of *M. leprae* and MAC with host Mϕs in terms of CK expression by Mϕs. In the present study,

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we have found some differential modes of CK mRNA expression of Mϕs infected with *M. leprae* or MAC.

MATERIALS AND METHODS

Microorganisms. *M. leprae* Thai-53 strain was harvested from infected footpads of BALB/c nude mice, and bacterial suspensions were prepared as follows (23). The infected footpads were homogenized in Hanks' balanced salt solution (HBSS) containing 5% fetal bovine serum (FBS) and centrifuged at 200 × *g* for 5 min. The upper layer was saved and the bacilli were collected by recentrifugation at 1500 × *g* for 15 min. The bacterial suspension was again centrifuged at 200 × *g* for 5 min, to remove bacterial clusters and the upper layer (about 90% volume) was harvested. The resultant bacterial suspension was then treated with 0.1 N NaOH for 1 min, neutralized with 0.1 N HCl, centrifuged at 1500 × *g* for 15 min. The resultant sediment was suspended into 10 ml of RPMI 1640 medium, pronase-P was added at 0.5 mg/ml, incubated at 37°C for 2 hr, and the pronase-P reaction was stopped by the addition of bovine serum albumin (BSA) at 0.7%. After washing twice with phosphate buffered saline (pH 7.2) containing 0.1% BSA by centrifugation, the resultant bacterial pellet was suspended into RPMI 1640 medium supplemented with 10% FBS. The viability of *M. leprae* organisms before and after pronase-P treatment was determined by the fluorescein diacetate/ethidium bromide (FDA/EB) staining according to the method of Tsukiyama, *et al.* (32). The percentage of green-stained viable cells in the *M. leprae* preparation before and after pronase-P treatment was estimated as 87.5 ± 1.2% and 86.2 ± 1.4% (n = 3). This indicates that pronase-P treatment did not cause severe damage in the cell surface of the *M. leprae* organisms.

Virulent SmT variant of MAC N-260 strain, showing smooth, transparent and irregularly-shaped colonies and avirulent SmD variant, showing smooth, opaque, and dome-shaped colonies (9) were used. MAC N-260 strain was identified as *M. intracellulare* by a DNA probe test, and determined to belong to serovar 16 by an agglutination test. MAC organisms were grown in Middlebrook 7H9 medium and bacterial suspensions prepared with 0.1% BSA-PBS were frozen at –80°C until use. Before use, the bacterial suspension was centrifuged at 200 × *g* for 5 min to remove bacterial clumps.

Expression of cytokine mRNA by *M. leprae*- or MAC-infected Mϕs. Mϕ monolayer cultures prepared by seeding 5×10^6 peritoneal cells of 8- to 12-week-old male BALB/c mice on 60 mm culture dishes were incubated in 5 ml of 10% FBS-RPMI 1640 medium at 37°C for 48 hr in a CO_2 incubator (5% CO_2-95% humidified air). After washing with Hanks' balanced salt solution (HBSS) containing 2% FBS, the Mϕs were cultured in 5 ml of the medium containing 1×10^7 bacilli/ml (CFU/ml for MAC) of test organisms at 37°C in a CO_2 incubator for up to 24 hr.

At intervals, cultured Mϕs were harvested and reverse transcription (RT)-PCR analysis of the mRNAs expression of test CKs (IL-12 p40, TNF-α, IL-10, TGF-β), inducible nitric oxide synthase (iNOS), and ICAM-1 in the Mϕs was performed as described previously (21) with slight modifications. Total RNA was isolated from the Mϕs using the ISOGEN kit (Nippon Gene Co., Toyama, Japan). After deoxyribonuclease-I (DNase-I) (GIBCO BRL Co., Rockville, Maryland, U.S.A.) treatment (1 unit DNase-I/μg of RNA sample) at room temperature for 15 min, the resultant RNA samples were reverse transcribed to the first chain of cDNA using oligo dT primers (GIBCO) and 200 units Superscript II reverse transcriptase (GIBCO) with the standard reaction mixture (20 μl): 1 × reverse transcriptase (RT) buffer (pH 8.3), 1 mM each dNTP including dATP, dCTP, dGTP and dTTP (GIBCO), and 2.0 units of ribonuclease inhibitor (GIBCO). After 1-hr reaction at 42°C and subsequent heating at 72°C for 15 min, 1 μl of aliquots of resultant cDNA were amplified specifically by PCR in the standard reaction mixture (50 μl) containing 1 × PCR buffer (pH 8.3), 0.2 mM of each dNTP, 1 unit of *Taq* polymerase (Takara Biomedicals Co., Tokyo, Japan), and 20 pmoles of sense and antisense primers for test proteins (synthesized by Greiner Labortechnik Co., Tokyo, Japan) as follows (sense/antisense): IL-12 p40 (CGT GCTCATGGCTGGTGCAAAG/CTTCAT CTGCAAGTTCTTG GGC); TNF-α (AG CCCACGTCGTAGCAAACCACCAA/AC

ACCCATTCCCTTC ACAGAGCAAT); IL-10 (TGACTGGCATGAGGATC AGCAG/ATCCTGAGGGTCTTCAGCTT); TGF-β1 (AGCCCTGGATACCAACTATTGCTTCA GCTCCACAG/AGGGGCGGGGCGGGG CGGGGCTTCAGCTGC); iNOS (CCTGC TCACTCAGCCAAG/AGTCATGGAGCC GCTGCT); and ICAM-1 (CAGGAGAGC ACAAACAGCAGTG/AGAGCGGCAGA GCAAAAGAAGC). Reactions were carried out in a DNA Thermal Cycler (ASTEC Corp., Fukuoka, Japan) for 25 cycles including denaturing at 94°C for 1 min, annealing at 58°C for 2 min, and extension at 72°C for 2 min for each cycle. PCR products were analyzed by electrophoresis on ethidium bromide-stained 2% agarose gels.

In some experiments, CK mRNA expression by Mφs was measured by the ribonuclease (RNase) protection assay using Riboquant™ Multi-Probe RNase Protection Assay System (Pharmingen Co., San Diego, California, U.S.A.) according to the manufacturer's instruction manual. In this study, mCK-2b and mCK-3 mouse cytokine multiprobe template sets were used for measurement of cytokine mRNAs as follows: mCK-2b: IL-10 and IL-12 p40 mRNAs; mCK-3: TNF-α and TGF-β mRNAs.

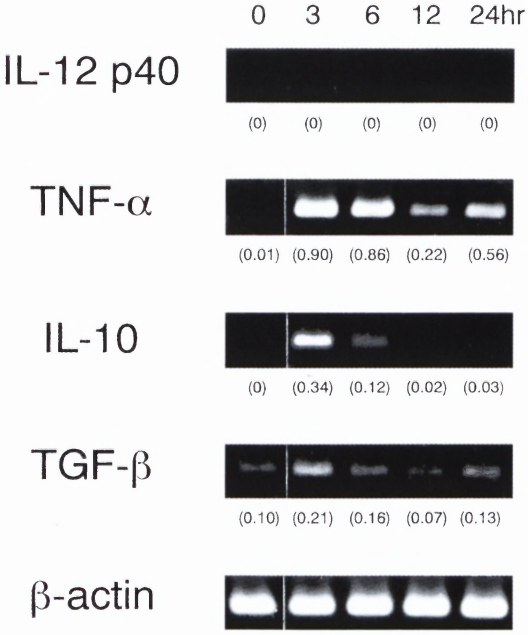

FIG. 1. Expression of IL-12 p40, TNF-α, IL-10, and TGF-β mRNAs by Mφ infected with *M. leprae*. The relative intensities of the RT-PCR bands of individual cytokines were calculated by normalizing to the intensity of the β-actin band. The values in parentheses are cytokine band/β-actin band ratios.

RESULTS

Expression of CK mRNAs by *M. leprae*-infected Mφs. The time-course of the expression of IL-12, a proinflammatory CK TNF-α, and immunosuppressive CKs (IL-10, TGF-β) in Mφs stimulated with *M. leprae* infection was examined by the RT-PCR assay. Fig. 1 shows the profiles of the mRNA expression of these CKs by Mφs during chase cultivation after *M. leprae* infection. IL-12 p40 mRNA expression was not observed for Mφs at least during 24-hr cultivation after infection. Second, TNF-α mRNA expression was remarkably increased at 3 hr after infection, and thereafter, gradually declined until 24 hr. The TNF-α mRNA expression was at a considerably high level even at 24 hr. Third, IL-10 mRNA expression was somewhat increased at 3 hr after infection, and, thereafter, rapidly ceased, reaching an undetectable level by 12 hr. Fourth, TGF-β mRNA expression was slightly increased at 3 hr after infection, followed by a gradual decrease reaching the normal level at 24 hr. Notably, the IFN-γ (500 units/ml) treatment yielded no obvious change in the modes of CK mRNA expression of *M. leprae*-infected Mφs (unpublished observation).

Expression of CK mRNAs by MAC-infected Mφs. Fig. 2 shows Mφ mRNA expression profiles measured by the RT-PCR assay of IL-12, TNF-α, IL-10, and TGF-β after infection with MAC organisms with different levels of virulence, a virulent SmT variant and an avirulent SmD variant. The mRNA expression of IL-12 p40 was increased in Mφs infected with MAC SmT variant as well as in those infected with MAC SmD variant at 3 hr after infection. The mRNA expression, thereafter, gradually decreased, and almost disappeared at 24 hr. Second, TNF-α mRNA expression was markedly increased at 2 hr after MAC SmT as well as MAC SmD infection, and, thereafter, gradually declined until 24 hr. The intensities of TNF-α mRNA expression of MAC SmT- and MAC SmD-infected Mφs were nearly identical. Notably, intense expression was still seen for the TNF-α

THE TABLE. *Expression of IL-12 p40, TNF-α, IL-10, and TGF-β mRNAs by Mϕs infected with MAC N-260 or* M. leprae *alone or co-infected with both organisms.*[a]

Cytokine	Time after infection (hr)	Relative intensity of mRNA expression of Mϕs infected with:		
		M. leprae	MAC	*M. leprae* and MAC
IL-12 p40	0	0[b]	0	0
	3	1.48	0.30	0.61
	6	0	1.19	0
	12	0	1.40	0
	24	0	0	0
TNF-α	0	0.58	0.58	0.58
	3	4.60	9.46	6.89
	6	4.86	2.71	4.42
	12	0.77	0.41	0.59
	24	0.25	0.09	0.30
	48	0.10	0.05	0.04
IL-10	0	0	0	0
	3	3.08	0.59	2.28
	6	0	0	1.05
	12	0	0	0
TGF-β	0	0.60	0.60	0.60
	3	1.27	1.36	1.08
	6	1.38	1.19	1.00
	12	0.34	0.48	0.60
	24	0.29	0.06	0.23
	48	0.12	0.12	0.13

[a] The relative intensity of each CK mRNA expression in terms of CK/L32 band ratios were estimated from the data indicated in Fig. 4.

[b] Undetectable.

mRNA even at 24 hr. Third, IL-10 mRNA expression was increased at 3 hr after MAC SmT and MAC SmD infection. The mRNA expression was retained at the same levels during 3 to 6 hr, and, thereafter, gradually declined until 24 hr. Notably, IL-10 mRNA expression during 3 to 6 hr was much stronger in MAC SmD-infected Mϕs than in MAC SmT-infected Mϕs. Fourth, TGF-β mRNA was constitutively expressed by uninfected Mϕs. Both MAC SmT and MAC SmD infections caused a moderate increase in TGF-β mRNA expression at 3 hr, and the increased level of TGF-β mRNA expression was retained until 24 hr. In separate experiments, the increase in TGF-β mRNA expression lasted at least for 48 hr (data not shown). Similar levels of TGF-β mRNA expression were seen for Mϕs infected with MAC SmT and those infected with MAC SmD. Notably, the IFN-γ (500 units/ml) treatment yielded no obvious change in the modes of CK mRNA expression of MAC N-260-infected Mϕs (unpublished observation).

Expression of CK mRNA by Mϕs co-infected with *M. leprae* and MAC. It has been reported that co-infection of MAC with *M. leprae* increases the pathogenicity of leprosy bacilli and facilitates the progression of the disease *in vivo* ([14]). Thus, it is of interest to examine the mode of CK mRNA expression by Mϕs co-infected with *M. leprae* and MAC. For this purpose, we measured the expression of IL-12, TNF-α, IL-10 and TGF-β mRNAs by Mϕs co-infected with both organisms by the RNase protection assay. As shown in Fig. 3 and The Table, Mϕs co-infected with both organisms showed the mRNA expression of IL-12, TNF-α, and IL-10 in an intermediate mode for those of Mϕs infected with either *M. leprae* or MAC alone. The levels of IL-12 and IL-10 mRNA expression seen in Mϕs infected with *M. leprae* alone were decreased due to co-infection with MAC, while the opposite phenomenon was observed for TNF-α mRNA expression. On the other hand, the level of TGF-β mRNA expression was somewhat decreased in Mϕs

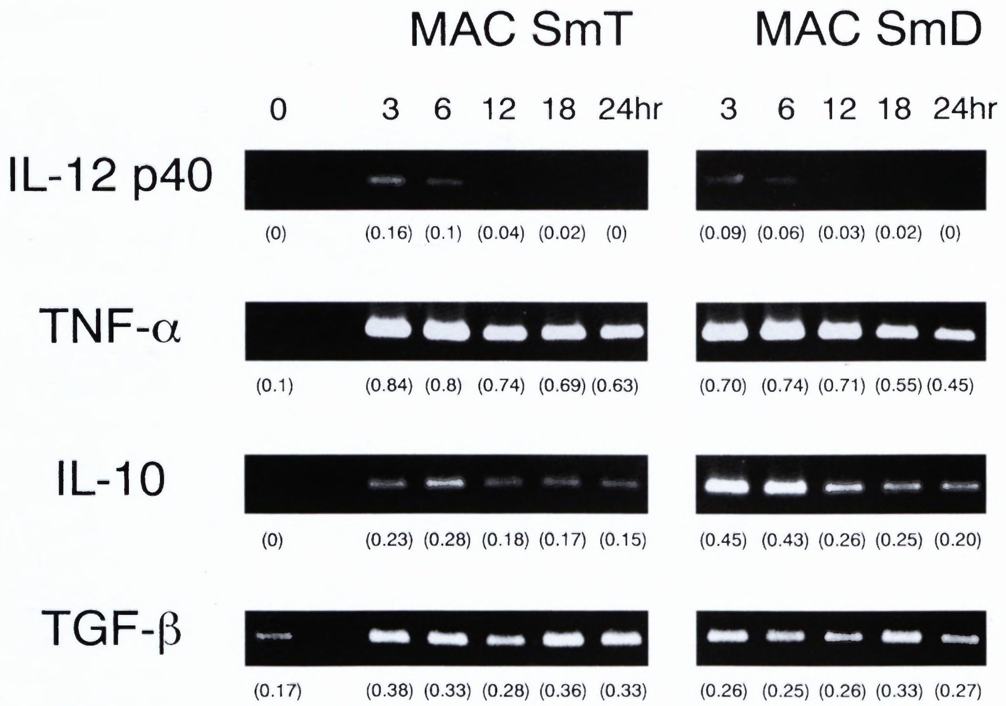

FIG. 2. Expression of IL-12 p40, TNF-α, IL-10, and TGF-β mRNAs by Mϕs infected with MAC SmT or SmD colonial variant. The other details are the same as those described in the legend of Fig. 1.

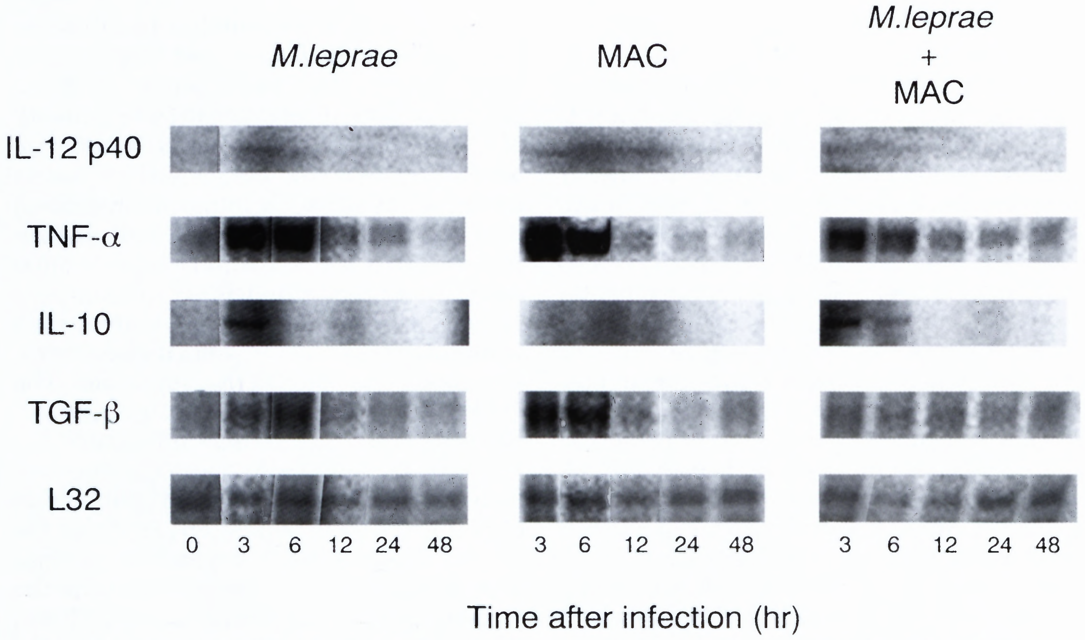

FIG. 3. Expression of IL-12 p40, TNF-α, IL-10, and TGF-β mRNAs by Mϕs infected with *M. leprae* or MAC alone or co-infected with both organisms. CK mRNA expression by Mϕs was measured by the RNase protection assay. The mRNA expression of L32, a housekeeping gene, was measured as the positive control of constitutive mRNA expression.

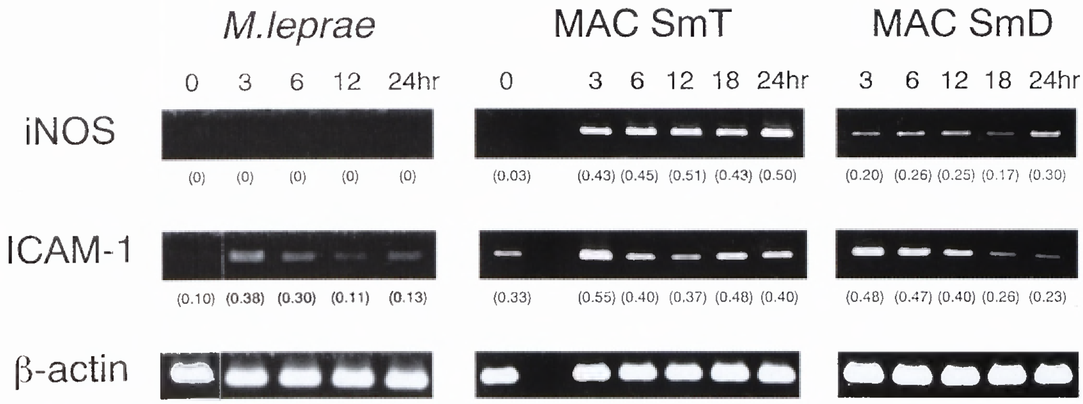

FIG. 4. Expression of iNOS and ICAM-1 mRNAs by Mφs infected with *M. leprae* or MAC. The other details are the same as those described in the legend of Fig. 1.

co-infected with both organisms as compared with those of Mφs infected with each organism alone.

Expression of iNOS and ICAM-1 mRNA by *M. leprae*- and MAC-infected Mφs. Fig. 4 shows Mφ mRNA expression profiles of iNOS and an adhesion molecule ICAM-1 measured by the RT-PCR assay, when Mφs were infected with either *M. leprae* or MAC. MAC infection caused a marked increase in the iNOS mRNA expression by Mφs at 3 hr post infection, whereas *M. leprae* infection failed to show such an effect. The increase in iNOS mRNA expression in MAC-infected Mφs lasted at least for 24 hr. Notably, MAC SmT-infected Mφs expressed stronger mRNA during 3 hr to 24 hr than did MAC SmD-infected Mφs. Second, ICAM-1 mRNA was constitutively expressed by uninfected Mφs. The mRNA expression of ICAM-1 was increased in Mφs at 3 hr after infection with *M. leprae*, as well as MAC SmT or MAC SmD, and, thereafter, gradually decreased until 24 hr. Similar levels of ICAM-1 mRNA expression were noted for MAC SmT- and MAC SmD-infected Mφs. These results on ICAM-1 mRNA expression are consistent with our previous findings concerning ICAM-1 expression in a protein level by *M. leprae*- and MAC-infected Mφs (23, 30).

DISCUSSION

The present study revealed some differences in the profiles of Mφ expression of CK and other protein genes between *M. leprae*- and MAC-infected Mφs, as follows. Although weak but significant increase in the IL-12 p40 mRNA expression was noted for MAC-infected Mφs in the early-phase (3 hr) after infection, such a phenomenon was not observed for *M. leprae*-infected Mφs. The latter finding is enigmatic, since the important role of Mφ-derived IL-12 in the establishment of Th1 response in *M. leprae*-infected hosts has been well documented (14, 16, 26, 29). Moreover, in the case of *M. leprae* infection in mice without immunodeficiency, bacterial growth at the sites of infection is well controlled due to strong expression of anti-*M. leprae* cellular immunity that is mediated by host Th1 cells in humans (14, 16, 29, 33) and in mice (2, 4, 13). It appears that this strange situation may be attributable to our experimental conditions, particularly that we used murine resident peritoneal Mφs. That is, efficient production of IL-12 by *M. leprae*-infected Mφs may required additional stimulatory signals other than phagocytosis of the organisms. It is likely that priming of Mφs with certain stimulants, that is deficient in resident Mφs, may be prerequisite for the gene expression of IL-12 induced by engulfment of *M. leprae* organisms. Concerning this point, further studies are needed.

In the case of MAC-infected Mφs, the increased levels of IL-10 mRNA expression at 3 hr were maintained for long time periods after infection (at least until 24 hr). On the other hand, in the case of *M. leprae*-infected Mφs, IL-10 mRNA expression, which was increased at 3 hr post infection, rapidly declined, reaching the undetectable level by 12 hr. However, *M. leprae* infection caused a considerably stable expression of TNF-α and ICAM-1 mRNA for 24 hr after infection. The instability of IL-10 mRNA expression in *M. leprae*-infected Mϕs may be due to rapid down-regulation of the IL-10 mRNA transcription after 3 hr, rather than due to rapid decay of IL-10 mRNA itself.

It has been proposed that immunosuppressing cytokines, such as IL-10 and TGF-β, play important roles in the establishment of persistent infections by pathogenic mycobacteria, including MAC and *M. leprae*, either by down-regulating Mϕ antimycobacterial functions (7, 8, 24, 29) or by attenuating mycobacteria-induced Mϕ apoptosis coupled with the elimination of intramacrophage organisms (5, 17). However, as indicated in Fig. 1, the mRNA expression of these cytokines was not stronger in Mϕs infected with virulent MAC (SmT variant) than in those infected with avirulent MAC (SmD variant). Notably, IL-10 mRNA expression of MAC SmT-infected Mϕs was conversely much weaker than that of MAC SmD-infected Mϕs. These findings suggest the possibility that neither IL-10 nor TGF-β may play crucial roles in the mechanisms for bacterial escape from Mϕ antimicrobial mechanisms. However, further *in vivo* studies are needed to reveal detailed profiles of the CK expression in granulomas of *M. leprae*-infected animals before such a conclusion is made.

Co-infection of MAC to *M. leprae*-infected mice is known to increase the pathogenicity of leprosy bacilli and facilitate the progression of the disease in mice (12). In the present study, it was found that Mϕs co-infected with both *M. leprae* and MAC showed the CK mRNA expression (except for TGF-β) in an intermediate mode of those of Mϕs infected with either *M. leprae* or MAC alone. This implies that the CK expression of *M. leprae*-infected Mϕs may be modified by co-infection with MAC. Notably, the IL-12 mRNA expression of *M. leprae*-infected Mϕs at 3 hr after infection was reduced to less than half of the original level (ca. 60% reduction) due to MAC co-infection. Moreover, Mϕs co-infected with both of these organisms failed to show durable expression of IL-12 mRNA, as observed in the case of Mϕs infected with MAC alone during 3 hr to 12 hr after infection. Thus, it is implicated that anti-*M. leprae* Th1 response of host animals may be hindered due to co-infection with MAC. This may account for the increase in the pathogenicity of *M. leprae*, when *M. leprae*-infected mice were further co-infected with MAC organisms (12).

Although MAC-infected Mϕs displayed markedly increased iNOS mRNA expression during 3 hr to 24 hr after infection, such an increase in the iNOS gene expression was not noted for *M. leprae*-infected Mϕs. Notably, reactive nitrogen intermediates, including nitric oxide radical produced by iNOS, play crucial roles in Mϕ antimicrobial mechanisms against mycobacteria, including *M. tuberculosis*, MAC, and *M. leprae* (1, 3, 9, 22, 30). It, thus, appears that the defect of *M. leprae* in inducing the iNOS expression by Mϕs phagocytizing the organisms plays a favorable role for the organisms in escaping from Mϕ antimicrobial mechanisms.

In summary, the present study revealed that there are noticeable differences in the modes of the mRNA expression of IL-12, IL-10, and iNOS in *M. leprae*-infected Mϕs, as compared to MAC-infected Mϕs. These findings may suggest differential interactions of *M. leprae* and MAC organisms with murine peritoneal Mϕs in terms of the activation of signal transduction pathways for expression of some kinds of immunoregulatory cytokines and immunoprotective enzymes. Further investigations are needed to elucidate the precise meaning of these findings, in particular the studies using peritoneal Mϕs, which are given priming with various stimulants before mycobacterial infection, or blood monocyte-derived Mϕs. On this point, further studies are currently under way.

SUMMARY

In the present study, we examined profiles of the interaction of *Mycobacterium leprae* and *Mycobacterium avium* complex (MAC) with murine peritoneal macrophages (Mϕs) in terms of up-regulation of Mϕ expression of proinflammatory and immunosuppressing cytolines (CKs) after infection. First, the reverse transcription polymerase chain reaction (RT-PCR) assay revealed that both MAC and *M. leprae* infections up-regulated Mϕ mRNA expression IL-12, TNF-α, IL-10, and transforming growth factor-β (TGF-β), except that *M. leprae*-infected Mϕs showed no increase in the IL-12 mRNA expression. Second, the RT-PCR assay also showed some differences between *M. leprae*- and MAC-infected Mϕs with respect to the modes of IL-10 and inducible nitric oxide synthase (iNOS) mRNA expression. That is MAC, but not *M. leprae,* infection caused a prolonged increase in the expression of IL-10 and iNOS mRNAs. Third, a ribonuclease protection assay revealed that Mϕs co-infected with MAC and *M. leprae* showed the IL-12, TNF-α and IL-10 mRNA expression in an intermediate mode of those of Mϕs infected with either *M. leprae* or MAC alone. This implies that the CK expression of *M. leprae*-infected Mϕs may be modified by co-infection with MAC. These findings may suggest differential interactions of *M. leprae* and MAC organisms with murine peritoneal Mϕs in terms of the activation of signal transduction pathways for expression of some kinds of immunoregulatory cytokines and immunoprotective enzymes.

RESUMEN

Se infectaron macrófagos peritoneales murinos con *Mycobacterium leprae* y *Mycobacterium avium* complejo (MAC), y se examinaron los perfiles de expresión de citocinas (CKs) proinflamatorias e inmunosupresoras consecuentes a la infección. Primero, la reacción en cadena de la transcriptasa reversa (RT-PCR) reveló que tanto la infección por MAC como por *M. leprae*, sobreactivaron la expresión de los mRNAs para IL-12, TNFα, IL-10, y el factor β del crecimiento transformante (TGF-β). También se observó, que a diferencia de los macrófagos infectados con MAC, los infectados con *M. leprae* no mostraron ningún incremento en la expresión del mRNA para IL-12. Segundo, el ensayo de RT-PCR mostró algunas diferencias entre los macrófagos infectados con *M. leprae* y MAC con respecto a los modos de expresión de los mRNAs para IL-10 e iNOS. Concretamente, la infección por MAC, pero no por *M. leprae*, causó un incremento prolongado en la expresión de los mRNAs para IL-10 e iNOS. Tercero, un ensayo de protección de la ribonucleasa reveló que los macrófagos co-infectados con MAC y *M. leprae*, mostraron un patrón de expresión de los mRNA para IL-12, TNFα, e IL-10, intermedio entre los patrones mostrados por los macrófagos infectados con los microorganismos por separado. Esto implica que la expresión de CKs por los macrófagos infectados con *M. leprae*, puede ser modificado por la co-infección con MAC. Los resultados sugieren que *M. leprae* y MAC interaccionan de manera diferente con los macrófagos murinos y que esto se refleja en la activación de diferentes vías de señalización relacionadas con la síntesis de citocinas inmunorreguladoras e inmunoprotectoras.

RÉSUMÉ

Dans cette étude, nous avons examiné les profils d'expression des cytokines (CKs) pro-inflammatoires et immunosuppressives issus de l'interaction de *Mycobacterium leprae* et du complexe de *Mycobacterium avium* (MAC) avec des macrophages murins (Mϕs), en terme d'augmentation de l'expression d'ARNm des CKs des macrophages après infection. Premièrement, l'essai de transcription inverse suivie de réaction de polymérase en chaîne (RT-PCR) a montré que l'infection tant par MAC et que par *M. leprae* augmentait l'expression de IL-12, TNF-α, IL-10 et du facteur de croissance induisant la transformation de type β (TGF-β), sauf que les macrophages infectés par *M. leprae* n'ont pas montré d'augmentation de l'expression de l'ARNm de l'IL-12. Deuxièmement, l'essai de RT-PCR a aussi montré quelques différences entre les Mϕs infectés par *M. leprae* et les Mϕs infectés par MAC en ce qui concerne les modes d'expression de l'ARN-m de l'IL-10 et de la synthétase inductible du monoxide d'azote (iNOS). Plus précisément, l'infection par MAC, mais pas *M. leprae*, provoque une augmentation prolongée des taux d'ARNm de l'IL-10 et de la iNOS. Troisièmement, un essai de protection contre la digestion par les ribonucléases a révélé que les Mϕs coinfectés par MAC et *M. leprae* montraient un niveau intermédiaire d'expression des ARNm de l'IL-12, de TNF-α et de l'IL-10 par rapport à ceux exprimés dans les Mϕs infectés par *M. leprae* ou MAC seuls. Ceci implique que l'expression des CKs des Mϕs infectés par *M. leprae* peut être modifiée par une coinfection avec MAC. Ces données suggèrent une interaction différentielle de *M. leprae* et des microorganismes du MAC avec les Mϕs péritonéaux de souris au niveau des voies de transduction des signaux intracellulaires pour l'expression de certaines cytokines immunorégulatrices et d'enzymes induisant une immunoprotection.

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