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Human Cytomegalovirus-Induced Interleukin-10 Production Promotes the Proliferation of *Mycobacteroides abscessus* subspecies *massiliense* in Macrophages

사람세포거대바이러스로 유도된 IL-10 생산에 의한 큰포식세포에서 *Mycobacteroides abscessus* subspecies *massiliense*의 증식 촉진

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ABSTRACT

Human Cytomegalovirus-Induced Interleukin-10 Production Promotes the Proliferation of *Mycobacteroides abscessus* subspecies *massiliense* in Macrophages

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Human cytomegalovirus (HCMV) is a β-herpesvirus and has a seroprevalence of approximately 60-80% of the world's population. In initial infections, the symptoms are minimal but it can establish a lifelong latent infection in the body. HCMV activity or a latent infection regulates the cell cycle and cytokines, which affects the host innate immune system and enables severe infection spreading within the human body. HCMV encodes immune evasion proteins such as US2, 3, 11 and UL111A which exacerbates the host immune response. In such a host immune-suppressive environment, there is a risk that the host immune system may not be able to adequately defend against pathogens. When HCMV controls the immune system of an infected host, other pathogens can infect the same host. As examples, HCMV and HIV or HCMV

and *Mycobacterium tuberculosis* co-infection cases have been reported. Therefore, this study investigated whether or not *Mycobacteroides abscessus* subspecies *massiliense* (*M. abscessus* subsp. *massiliense*) is more likely to induce co-infection under HCMV latent infection.

M. abscessus subsp. *massiliense*, a rapidly growing *Mycobacterium* (RGM), is the most drug-resistant pathogen among the nontuberculous mycobacteria (NTM). It is uncertain whether HCMV infection itself increases the risk of *M. abscessus* subsp. *massiliense* infection or a predisposition to HCMV viremia is the bacterial disease factor. As demonstrated by transcriptomic analysis, HCMV infection suppressed the regulatory pathways of interferon-gamma (IFN-γ), tumor necrosis factor-alpha (TNF-α), and interleukin-1 (IL-1), inhibiting the immune responses to *M. abscessus* subsp. *massiliense* co-infection in macrophages. In addition, the HCMV infection cycle regulates the IL-10 signaling cascade of the host cell. It was confirmed that cmvIL-10 signals and binds to receptors in a manner similar to host IL-10. Macrophage increased IL-10 secretion when infected with HCMV, in IL-10 dependent manner and promoted *M. abscessus* subsp. *massiliense* proliferation. When the cmvIL-10 produced after virus infection was removed, the production of host IL-10 reduced, and if the host IL-10 was neutralized, I found that the bacterial proliferation reduced. HCMV infection increases IL-10 secretion and provides a basis for the host immune response in the context of pathogenic *M. abscessus* subsp. *massiliense* co-infection.

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ABBREVIATIONS

AA: amino acid

ATCC: American Type Culture Collection

CD: Cluster of differentiation molecule

CFU: colony-forming unit

CIP: Collection of Institute Pasteur

CLEC: c-type lectin

CMV: cytomegalovirus

CPE: cytopathic effect

Ct: cycle threshold

DC: Dendritic cell

DNA: deoxyribonucleic acid

DAPI: 4,6-diamidno-2-phenylindole

DMEM: Dulbecco's Modified Eagle Medium

EGFP: Enhanced Green Fluorescent Protein

ELISA: enzyme-linked immunosorbent assay

FACS: fluorescence-activated cell sorter

FBS: fetal bovine serum

FITC: fluorescein isothiocyanate

GEO: gene expression omnibus

GFP: green fluorescence protein

GO: gene ontology

GPL: glycopeptidolipid

GSEA: gene set enrichment analysis

HCMV: human cytomegalovirus

HCT: hematopoietic stem cell transplantation

HIV: human immunodeficiency virus

HLA: human leukocyte antigen

hsp65: heat shock protein 65

IE: immediate-early (phase of cytomegaloviral gene expression)

IFN: interferon

Ig: immunoglobulin

IL: interleukin

kb: kilobase

NF-κB: nuclear factor kappa-light-chain-enhancer of activated B

cells

NLRP: nucleotide-binding oligomerization domain, leucine rich repeat and pyrin domain containing

NTM: nontuberculous mycobacteria

MAB: *Mycobacteroides abscessus*

MEM: minimum essential media

MFI: mean fluorescence intensity

M.O.I: multiplicity of infection

ORF: open reading frame

PBMC: peripheral blood mononuclear cell

PBS: phosphate buffered saline

PCR: polymerase chain reaction

PFU: plaque forming unit

PI(3)K: phosphatidylinositol 3,4,5 triphosphate kinase

qPCR: quantitative polymerase chain reaction

RNA: ribonucleic acid

RPMI: Roswell Park Memorial Institute

RT-PCR: reverse transcriptase polymerase chain reaction

TGF: tumor growth factor

Th: threshold

TLR: toll-like receptor

TNF: tumor necrosis factor

UL: unique long domain

US: unique short domain

INTRODUCTION

1. Characteristics of human cytomegalovirus

Human cytomegalovirus (HCMV), which is a member of the *Cytomagalovirus* genus of the *β-herpesvirinae* subfamily of the *Herpesviridae*,[1] infects people of all ages, more than 60-80% of the world's population.[2] HCMV was first isolated in the 1956.[3] The economic burden of CMV-congenital infections reaches $1.9 billion annually in the USA with about $360 million invested in vaccine development.[4]

HCMV consists of a lipid icosahedron envelope, has a diameter of 150-200 nm, a length of approximately 235 kb, the dielectric is linear, and is unsegmented. HCMV is a genetically diverse virus with approximately 200 open reading frames (ORFs) producing effector proteins.[5] DNA replication follows the bidirectional replication model of double-helix DNA. HCMV has many genes related to proteins necessary for replication, evasion of immune mechanisms, induction and regulation of inflammatory responses, and regulation of the cell cycle.

The nucleic acid of HCMV is located in the center, enclosed by a capsid, matrix protein and envelope, which is a protein layer and cell membranes derivatives.[3] Virus replication takes place in the nucleus and has a lysogenic cycle. Virus-infected cells are destroyed to form plaques, and in some cases, they induce the cytopathic effect (CPE) without lysing the cells. After infecting the cells, virus replication takes place within 12 h and CPE persists for approximately 7-14 days.[6]

Invasion of a host cell consists of the viral glycoprotein binding to a receptor on the membrane of the host cell and causing

intracellular translocation. Humans and monkeys are the hosts, and transmission is through contact and body fluids, such as urine, saliva, blood semen and breast milk.

The virus is transmitted by vertical transmission after maternal primary cytomegalovirus infection and at a young age.[7] In the case of primary infection, symptoms are usually mild and induce a latent infection. If the virus is present in the human body in the form of a latent infection,[8] the effect of the antiviral immune response may not be significant; as a result, a persistent infection could be maintained. Infection with HCMV could cause infectious fatigue syndrome, fever, sore throat, mononucleosis-like symptoms, pneumonia and dysfunction of several organs including the pharynx, esophagus, respiratory system, eyes and liver.[9; 10]

In many species, most CMV infections are asymptomatic (no signs) but immunosuppressed patients, and immunocompromised individuals such as people with AIDS may be symptomatic. The association of the immune response and the virus with disease progression in people who are continuously infected with CMV is still poorly understood.

Various cells act as reservoirs for CMV, which include myeloid cells, monocytes, fibroblasts, smooth muscle cells, epithelial and endothelial cells.[11] Macrophages and endothelial cells have an important role in the host cells in a latent infection.[12; 13]

2. Characteristics of *Mycobacteroides abscessus* subspecies *massiliense*

Mycobacteroides abscessus (MAB)[14] was first isolated in the 1950s,[15] and has steadily increased over the past 20 years. The MAB complex is a rapidly growing *Mycobacterium* (RGM) that leads

to nosocomial infection. Colony formation is after 7 days of culture in an agar medium, *M. abscessus*, following *Mycobacterium avium*, is the dominant agent of NTM causing lung disease.[16; 17; 18] The MAB complex is classified into two subspecies: *M. abscessus* subsp. *abscessus,* the former species *M. abscessus* and *M. abscessus* subsp. *bolletii*. *M. abscessus* subsp. *bolletii* was suggested to combine the two species, *M. massiliense* and *M. bolletii*.[19; 20; 21] *M. abscessus* subsp. *massiliense* can be classified into two types (I and II) using the *hsp65* sequence analysis.

MAB is divided into two types: non-cord-forming smooth colony (MAB-S) and cord-forming rough colony (MAB-R), in which the glycopeptidolipid (GPL)[22; 23; 24] on the cell wall components is caused by the loss or mutation of the GPL biosynthesis-related gene. The GPL discerns 31 serotypes among MAB, and relates to immunopathological responses during infection.[25]

The MAB-S type has an effect on survival through GPL-related biofilm formation and inhibits bacterial-induced cell death.[26; 27] GPL-related biofilm is important to resist physicochemical stress, antimicrobials and immune defense mechanisms, establishing their persistence.[28] The MAB-R type is noted to be more virulent and associate with acute respiratory disease. The MAB-R induces stronger immune responses than the MAB-S, and the MAB-R survives more effectively in macrophages and dendritic cells.[29]

MAB complex is not only highly virulent with diverse susceptibility patterns to many antibiotics but it is also the most difficult to treat.[30] The phenotypes are very similar, but the heterogeneity between the strains is very large, and the difference in the base sequence of the antibiotics resistance-related gene is

clear. MAB lung disease is also resistant to many antibiotics, lacks effective oral antibiotics and is difficult to treat;[31; 32] despite the long-term antibiotic treatment, including months of injection treatment, the treatment outcomes are poor.[33; 34] MAB complex can cause lung disease, lymphadenitis, skin and soft tissue osteoarthritis and disseminated disease, of which lung disease accounts for more than 90% of cases.[31] Transmission of MAB complex begins when bacteria that are primarily present in the surrounding environment infect the respiratory tract through the air,[35; 36] similar to TB. Symptoms are nonspecific and varied, and most of the patients suffer from a recurrent or chronic cough due to respiratory symptoms.[31; 37] The distribution of bacteria that cause NTM diseases varies from region and differs in countries.[38]

MAB lung disease shows two characteristic forms, cystic fibrosis and nodular bronchiectasis,[39; 40] and the effective drug and therapeutic response differ depending on the subspecies.[41] Thus, it is important to confirm the exact subspecies before treatment.

In particular, *M. abscessus* subsp. *massiliense* shows a good therapeutic response to macrolide antibiotics, whereas most MAB shows an induced resistance to macrolides, thus the two can be differentiated.[38; 42; 43] The overall treatment period is to ensure that a negative conversion state is maintained for at least 12 months after successfully reaching negative conversion. However, to date, the overall outcome for the treatment of NTM lung disease has been unsatisfactory, especially for MAB lung disease.[44] NTM including MAB complex is often untreated and sometimes referred to as an "incurable nightmare". Long-term treatment of NTM brings a high burden and serious side effects to patients and causes a

financial burden to society. In a 2010 report, a total of $855 million was spent in relation to NTM in the United States.[45; 46] According to the WHO data, on the global tuberculosis eradication project, although the death rate from tuberculosis is gradually decreasing,[47] the NTM infection number of patients has increased recently, the mortality rate due to NTM is increasing.[48] More than 200 species of *Mycobacterium* have been isolated to date, and NTM are composed of the remaining *Mycobacterium* except for the tuberculosis-causing *Mycobacterium*.

The incidence and prevalence of NTM lung disease tend to increase worldwide, and the frequency, incidence, and prevalence of MAB complex isolation from clinical specimens are increasing rapidly in Korea as well.[49; 50; 51] In particular, Korea, unlike other countries, has a very high incidence of lung diseases caused by MAB complex[52; 53] and skin diseases are expected to increase.

3. HCMV infection and immune modulation

HCMV activity or a latent infection regulates the cell cycle and cytokines, which affects the innate immune system of the host, and enables severe infection spreading within the human body. HCMV encoded immune evasive proteins alter the immune signaling and activation of the host.[54] The protein product of the HCMV US11, US2 and US3 downregulate the expression of the major histocompatibility complex (MHC) class I and II molecules on cells, such as dendritic cell, macrophage, B cell and antigen-presenting cells, which blocks the presentation of HCMV-mediated peptides bound to MHC molecules.[55] Therefore, it regulates the recognition of T cells, reducing the role of T cells.

The HCMV UL16 and UL142 proteins block the ligand expression of activated NK cells on the surface of infected cells,[56; 57] whereas the HCMV UL83 protein can bind directly to NKp30, a receptor of activated NK cell receptor.[58] In this way, HCMV inhibits the activation of NK cells. Furthermore, the HCMV US18 and US20 proteins induce the degradation of MICA (a major stress protein) and prevent NK cells from recognizing signals from the infected cell.[57]

Virus-infected cells can prevent the spread of the virus through apoptosis. HCMV-infected cells inhibit programmed cell death to promote a favorable environment for viral replication. HCMV UL36 and UL37 proteins prevent apoptosis of infected cells, enhancing virus dissemination within the human body.[59; 60]

4. Features of HCMV IL-10

Cytomegalovirus-encoded cellular IL-10, which is a chemokine receptor homolog, has a partial role in enabling HCMV effectively infect the host and cause disease.[61] cmvIL-10 is the most important immunomodulatory system that suppresses and regulates excessive immune activation.[62; 63; 64] Inhibition of pro-inflammatory cytokines by IL-10 reduces the secretion of TNF-α, IL-1β, IL-6 and type I IFN.

cmvIL-10 is encoded and expressed by the *UL111A* gene, and HCMV infects monocytes to produce the gene. The UL111A is transcribed with a 175-amino acid cmvIL-10 protein during lytic infection.[65; 66; 67] When cmvIL-10 binds to the receptor, a cascade of intracellular signal transduction occurs. It was confirmed that cmvIL-10 signals and binds to receptors in a manner similar to host IL-10.[65]

Phosphatidylinositol 3-kinase (PI3K) / signal transducer and activator of transcription 3 (STAT3), are known to be regulated by IL-10.[56; 68] It translocates into the nucleus and activates various promoters.[69] Inhibition of pro-inflammatory mediators by cmvIL-10 was also demonstrated in other immune cells. Examples include the suppressed synthesis of type I interferon by plasmacytoid dendritic cells and the inhibition of the pro-inflammatory CXC chemokine ligand 10 (CXCL10) by microglia macrophages.[70] In addition, cmvIL-10 appears to stimulate the proliferation and differentiation of B lymphocytes and macrophages.

5. Objectives: HCMV co-infection with *M. abscessus* subsp. *massiliense*

According to recent reports, allogenic hematopoietic stem cell transplantation (HCT) or solid organ transplantation frequently causes MAB subspecies infection and very often lung disease, especially in Korea.[71; 72] It is uncertain whether CMV infection itself increases the risk of MAB subspecies infection or a predisposition to CMV viremia is bacterial disease factor.[72]

In this study, I investigated how the defense mechanism of the host cell is induced when *M. abscessus* subsp. *massiliense* simultaneously co-infects in a body with latent HCMV infection. Therefore, there is a phenomenon that macrophages, which are host defense cells, have to defend against them, but cannot suppress them well, so, the study tried to explain the mechanism that has not been revealed yet.

Macrophages infected with HCMV are considered as a co-infection situation, therefore, the immune system secretes the most potent anti-inflammatory cytokines.[73; 74] *M. abscessus* subsp.

massiliense is an intracellular bacterium and proliferates predominantly within macrophages.[75] Recent reports have confirmed that these tuberculosis and HCMV infections are mechanically significant;[76; 77] they have described the convergent epidemiology. However, studies on the etiology of HCMV and NTM interaction are to be in the absence of evidence. Therefore, I sought to determine the effects of proliferation and how HCMV-mediated immune regulation is achieved in the case of *M. abscessus* subsp. *massiliense* and co-infection, which is one of the MAB species, due to HCMV infection.

MATERIALS AND METHODS

1. Cell and Viruses

HEL299 (ATCC CCL-137) cells were cultured in Dulbecco's minimal essential medium (DMEM) with 10% fetal bovine serum (FBS) and 10% penicillin/ streptomycin. HEL299 cells were infected with the HCMV Towne (ATCC VR-977) and UL32-EGFP-HCMV-TB40E (ATCC VR-1578) strains, (Approved IBC No. R120523-1-1) and after culturing for 7-10 days, the medium was collected.[78; 79]

Viruses collected from the same batch were used for all experiments. Virus copy number was determined using quantitative polymerase chain reaction (qPCR). *M. abscessus* subsp. *massiliense* CIP (Collection of Institute Pasteur, Paris, France) 108297 strain was obtained from CIP (Approved IBC No. R170901-1). *M. abscessus* subsp. *massiliense* was grown in Middlebrook 7H9 broth (BD Biosciences) supplemented with 0.2% glycerol (Sigma-Aldrich), 10% oleic acid-albumin-dextrose-catalase (OADC; Fisher Scientific), and 0.05% Tween 80 (Sigma-Aldrich). Cultures were incubated at 37℃ with constant shaking (150 rpm) overnight. *M. abscessus* subsp. *massiliense* concentration was determined by measuring the optical density of 0.5-0.7 at 600 nm (OD600). Culturing for 2 days, mycobacteria were collected using centrifugation and homogenization. Frozen bacteria were stored at -80℃.

The human acute monocytic leukemia THP-1 (ATCC TIB-202) cells were cultured in RPMI 1640 media with 10% FBS(Gibco), 2 mM L-glutamine, 100 U/ml Penicillin, 100 μg/ml Streptomycin at 37℃ with 5% CO_2. THP-1 cells were differentiated using PMA 50

ng/ml, and after 24 h, they were exchanged for media without 1% PS to differentiate, and 2 days later, HCMV Towne and TB40 strain were infected with MOI of 10. 3 h after incubation, culture media were changed. 24 h after infecting HCMV, *M. abscessus* subsp. *massiliense* were infected with MOI of 2 and incubated for 1 h. Infected cells were washed twice with PBS, the media was changed. Changed in complete media without antibiotics for 1-3 days.

Human peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors (Approved IRB No. C-1306-0210494) using Ficoll-Hypaque Plus (GE Healthcare) gradient centrifugation. Isolated PBMCs were seeded and infected with HCMV at a MOI of 10 for 24 h, and the media was changed to establish HCMV infection. *M. abscessus* subsp. *massiliense* was infected at (MOI = 2) 24 h post-HCMV infection.

For colony-forming units (CFUs) assay, the infected cells were washed and detached with PBS containing 0.1% Triton X-100 (Sigma-Aldrich) and plated on LB agar.

2. Quantitative Genomic PCR

For amplification of HCMV *US17*, a control plasmid was prepared using pBHA vector contained a primer sequence and a probe sequence. Plasmid was obtained commercially (Bioneer), and the forward primer, reverse primer, and probe are configured as shown in Table 1. The evaluation test for the linear detection potential of the reference target was independently performed three times with a 10-fold serially diluted plasmid. qPCR was performed under universal conditions: 50℃ for 2 min, 95℃ for 10 min then 40 cycles of 95℃ for 15 sec, 55℃ for 15 sec, and 55℃ for 1 min by using QuantStudio 5 (Applied Biosystems).

PCR proceeded up to 40 cycles, and the degree of fluorescence was measured according to the number of cycles, and a threshold line (Th) was selected for the region where the fluorescence signal was exponentially amplified. The calculation of the standard curve was performed by regression analysis of the number of copies of the critical period value (Ct) and the value line intersection of the amplification curve.

3. Virus Titration

Representative virus vials were thawed and titers were measured using a conventional plaque assay or immune-staining assay. HEL299 cells were seeded into 24-well plate and incubated overnight in DMEM with 10% FBS at 37℃ with 5% CO_2. A total of 200 μl of diluted virus mixture were added to each well containing a monolayer of HEL299 cells. The virus mixture was removed after 2 h of incubation, washed two times with PBS. The cells were overlaid with opti-MEM containing 2% FBS and 1.5% of methyl cellulose (Sigma-Aldrich, USA) and incubated at 37℃ in 5% CO_2 for one day. The cells were fixed with methanol (Merk, USA) for 20 minutes at room temperature. Primary antibody (anti IE-1, a gift from E-S Huang)[80] was added to each well. The cells were incubated for 1 h. The cells were then incubated for 1 h with secondary antibody (anti-mouse IgG HRP, Thermo Fisher) at room temperature. The plate was washed and stained with Trueblue peroxidase substrate (KPL, USA).

4. Recombinant Proteins and Neutralizing Antibodies

Recombinant cmvIL-10 was purchased from R&D systems. Purified viral HCMV IL-10 antibody (cIL-10 antibody, polyclonal

goat IgG, R&D Systems) and human IL-10 antibody (IL-10 antibody, clone 23738, R&D Systems) were used at 10 μg/mL to neutralize cmvIL-10 and human IL-10 protein. Human IL-10 receptor alpha neutralizing monoclonal antibody (IL-10R NAb, clone 37607, R&D Systems) was used at 10 μg/mL. Corresponding isotype controls for neutralization experiments were obtained from R&D Systems.

5. Immunofluorescence Assay

Cells infected with HCMV were maintained in complete RPMI 1640 containing 2% heat-inactivated FBS and incubated for 1~7 days at 37℃. Virus-infected cells were washed twice with PBS, fixed in 4% paraformaldehyde (PFA) for 15 min and treated with 0.1% Triton X-100 for 5 min. Cells were then washed with PBST and reacted with the primary antibody (anti-IE1), followed by a Alexa 594-conjugated anti-mouse IgG antibody (Thermo Fisher), at room temperature. Fluorescent signals were observed using a Leica TCS SP8 Confocal Microscope (Leica Microsystems).

6. Flow Cytometry

The HCMV TB40E-infected THP-1 cells and PBMCs were collected and fixed with 4% paraformaldehyde for 20 minutes. The cells were washed 3 times with FACS buffer (PBS with 0.1% fetal bovine serum). The antibody used anti-human CD45 (HI30), CD3 (HIT3a), CD19 (HIB19), CD11b (ICRF44) and CD14 (MfP9) all purchased from BD Biosciences. Data were acquired on BD LSR Fortessa X-20 and analyzed using Flow Jo software, version (Tree Star, Ashland, OR).

7. Gene Expression Analysis and RNA Sequencing

Total RNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer's protocols. Messenger RNA was reverse transcribed into cDNA using a reverse transcription kit (Enzynomics). Quantification of *UL111A* and *IL-10* was performed in ABI PRISM 7900 (Applied Biosystems) using TaqMan PCR PreMix or SYBR Green PCR PreMix (Enzynomics). The primer sequences used were as follows: *UL111A* forward: 5′-TGT TGA GGC GGT ATC TGG AGA-3′; *UL111A* reverse: 5′-CCG TCT TGA GTC CGG GAT AG-3′; *UL111A* probe: 5′-CCG GTT TCC CGC AGG CGA CC-3′; *IL10* forward: 5′-GCC TAA CAT GCT TCG AGA TC-3′; *IL10* reverse: 5′-TGA TGT CTG GGT CTT GGT TC-3′; *β-actin* forward: 5′-GTT GCT ATC CAG GCT GTG-3′; *β-actin* reverse: 5′- TGA TCT TGA TCT TCA TTG TG-3′. After extraction of total RNA, for RNA sequencing analysis, the library preparation was performed using the NGS service provided by Ebiogen Inc. (Seoul, South Korea). 500ng of total RNA was prepared, and oligo-dT primer containing an Illumina-compatible sequence at the 5′ end was reacted with RNA to proceed with reverse transcription. Once the RNA template was degradated, the synthesis of the second strand was performed using random primers containing compatible linker sequences. To remove all reaction components, the double-stranded library was purified using magnetic beads. The library required for cluster generation was amplified to add the entire adapter sequence. The completed library was purified from the PCR product. High-throughput sequencing was proceeded as single-end 75 sequencing using NextSeq 500 (Illumina). After alignment with Bowtie2, QuantSeq3′ mRNA-Seq reading was performed.[81] Bowtie2 indices were

generated to align genome assembly sequences or genomes and transcripts in representative transcript sequences.

Transcripts were assembled through alignment files, their abundance was estimated, and differential expression of genes was detected. Differentially expressed genes were determined based on counts from unique and multiple alignments using coverage in Bedtools.[81].

The RC (Read Count) data were processed based on the quantile normalization method using EdgeR in R based on Bioconductor.[82] Gene classification was performed by the search method proceeded by DAVID1, and genes that were altered at least 4 fold (p<0.01) were analyzed for enrichment of gene ontology (GO) biological processes. MeV software was used for hierarchical clustering and GO analysis. Euclidean distance and average linkage clustering were applied to obtain hierarchical clustering. Enriched terms that passed FDR < 20% were selected.

For the Gene Set Enrichment Analysis (GSEA) for gene expression data, Molecular Signatures Database (V7.0) was used established on computing overlaps with GO gene sets (C5), obtained from the Broad Institute. All sequencing data can be searched at the Gene Expression Omnibus (GEO) database (GEO accession number: GSE141236).

8. ELISA

Infected cell culture supernatants were assessed for human IL-10 secretion using ELISA Duoset system (R&D Systems) according to the manufacturer's instruction. Culture supernatants collected by centrifugation at 587 g for 5 min to remove particulates, and stored at −80℃ until ELISA was performed.

9. Statistical Analysis

All data are presented as mean values ± standard error of measurement (SEM) of three independent determinations. The Student's t-test and one-way ANOVA test were performed to determine statistically significant differences between groups using the GraphPad Prism 6 (GraphPad Software). Bonferroni's or Tukey's multiple comparisons test between all possible combinations were performed as post-tests for one-way ANOVA, respectively. A value of $p<0.05$ was deemed to be statistically significant, represented by * $p<0.05$; ** $p<0.01$, and *** $p<0.001$.

RESULTS

Confirmation of HCMV infection in THP-1 differentiated macrophage

Three days after treating THP-1 differentiated macrophages with PMA, HCMV Towne was infected with an MOI of 4, and the CPE of the cells was observed. The state of the cells 24 and 72 h after infection was observed using a microscope (Figure 1A), and the expression of viral genes in the culture medium were confirmed using qPCR. I found that the IE-1 gene increased after 24-72 h had passed. The position of the IE-1 protein levels in cells was confirmed using a fluorescence microscope (Figure 1B). Collectively, these results demonstrated that it was mainly expressed in the cytoplasm 4 h after infection, and was located in the whole cell 24 h later.

Proliferation of HCMV TB40E in THP-1 differentiated macrophages and PBMCs

Cells were infected with representative two strains, fibroblast tropic strain Towne and epithelial/endothelial tropic strain TB40E; TB40E with the green fluorescent protein (GFP). Three days after treating THP-1 differentiated macrophages with PMA, HCMV TB40E was infected with MOI of 10, and then GFP was observed under a fluorescence microscope. From the result, GFP formed dots in the nucleus 3 h after the infection, and 24 h later, it was expressed in the whole nucleus as shown in Figure 2. Viral copy number using qPCR showed that HCMV was effectively replicated in THP-1 differentiated macrophages. Analysis via GFP using flow cytometry in THP-1 differentiated macrophage (Figure 2D) and human PBMCs monocytes (Figure 3A, B). Isolated PBMCs from healthy blood donor and infected with HCMV TB40E; expression of GFP analyzed every 72 h. Cell subsets can be distinguished by flow cytometry based on the expression of the cell surface markers. HCMV-infected monocytes were significantly different at 24 h with the following MFI. Collectively, these results show that the number of virus was increased in HCMV TB40E infected THP-1 differentiated macrophages and PBMCs.

HCMV promotes *M. abscessus* subsp. *massiliense* proliferation

The study investigated the effect of the proliferation of *M. abscessus* subsp. *massiliense* on HCMV TB40E-infected macrophages (MOI = 10). After infecting *M. abscessus* subsp. *massiliense* with MOI of 2, the same results as the HCMV Towne strain-infected macrophages were confirmed 3 h later. However, after 24 h, HCMV infected macrophages increased the number of intracellular bacteria, and after 72 h, fluorescence microscopy was used to confirm that the number of fluorescent particles in the cells increased. In addition, when the colony-forming units of *M. abscessus* subsp. *massiliense* were confirmed from the cell lysates, the number of bacteria increased (Figure 4A, B, D). The single infection and co-infection groups were analyzed via GFP using flow cytometry (Figure 4C).

Analysis of transcriptome profiles of HCMV-infected cells, *M. abscessus* subsp. *massiliense*-infected cells, HCMV/ *M. abscessus* subsp. *massiliense* -infected cells and control

Next, I analyzed the profiles of HCMV infected cells (MOI = 10), *M. abscessus* subsp. *massiliense* infected cells (MOI = 2), and HCMV/ *M. abscessus* subsp. *massiliense*-infected cells (Figure 5A). First, the HCMV-infected diet showed 880 differentiating genes (DEGs, 500; upregulated, 380; downregulated) compared to control macrophages (Table 2). Hierarchical clustering analysis showed a high similarity between HCMV- and HCMV/ *M. abscessus* subsp. *massiliense*-infected cells compared to *M. abscessus* subsp. *massiliense*-infected cells (Figure 5A). Based on these DEGs, I obtained hierarchical congestion between the four groups and found that *M. abscessus* subsp. *massiliense* co-infection did not totally alter transcription of HCMV-infected macrophages (Figure 5B). HCMV replication from macrophages results in a low immune responsiveness following *M. abscessus* subsp. *massiliense* infection, which is clearly demonstrated by GO analysis. According to the results obtained from DAVID software, *M. abscessus* subsp. *massiliense* single-infected macrophages contained an enriched GO pathway that included the cellular response to lipopolysaccharide (LPS) and TNF/ IL-1 compared to the enriched GO pathway in the co-infected group (Figure 5C). Gene Set Enrichment Analysis (GSEA) showed similar results in enriched GO pathways in which *M. abscessus* subsp. *massiliense* single-infected macrophages contained a positive regulation of immune response compared to co-infected groups.

To support these results, the innate, adaptive and antigenic GO

pathways were enriched with downregulated DEGs of HCMV-infected macrophages compared to control macrophages. Representative innate immune response genes such as TLRs, NLRP3, CLECs, HLAs and CDs are strongly regulated and anti-inflammatory when infected with HCMV (Figure 5D).

Confirmation of *UL111A* gene expression in HCMV-infected THP-1 differentiated macrophages

To confirm that HCMV promotes *M. abscessus* subsp. *massiliense* proliferation in the macrophages using IL-10 signaling, qPCR was used to test the predecessor of the *UL111A* gene encoding cmvIL-10 in HCMV-infected macrophages. Three days after treating THP-1 differentiated macrophages with PMA, HCMV TB40E was infected with MOI of 10, at 24 h prior to *M. abscessus* subsp. *massiliense* infection (MOI = 2). As expected, *UL111A* transcription was induced 12 h after HCMV infection (Figure 6).

HCMV infection regulates host IL-10 synthesis

Three days after treating THP-1 differentiated macrophages with PMA, HCMV TB40E was infected with MOI of 10, at 24 h prior to *M. abscessus* subsp. *massiliense* infection (MOI = 2). The expression of host IL-10 gene induced by HCMV infection in THP-1 macrophage and PBMCs was confirmed using qPCR and by measuring host IL-10 protein in the THP-1 macrophages and PBMCs culture medium using ELISA (Figure 7). In accordance with this, HCMV infection significantly induced host IL-10 synthesis in all the hosts of the two groups.

Viral IL-10 of HCMV induces host IL-10 synthesis in THP-1 macrophages and PBMCs

It has been confirmed that host and human IL-10 were synthesized by treating recombinant cmvIL-10 (Figure 8) or by culture supernatant of macrophages infected with HCMV (Figure 9). The expression of the IL-10 gene was confirmed using qPCR and by measuring IL-10 protein from the THP-1 macrophage and PBMCs culture medium using ELISA. PBMCs were treated with 200 ng/mL of cmvIL-10 for 6 h and 48 h, after harvesting. Collectively, these findings demonstrate that the gene expression and protein levels increased.

Host IL-10 is present when a neutralizing antibody of cmvIL-10 (cIL-10 NAb) is present at the time of HCMV infection. I found that the synthesis of host IL-10 reduced significantly (Figure 10). These results show that cmvIL-10 was secreted by HCMV-infected macrophages.

In particular, the expression level of host IL-10 did not change even when HCMV-infected macrophages and *M. abscessus* subsp. *massiliense* were co-infected. The expression levels of IL-10 synthesized by HCMV-infected macrophages co-infected with *M. abscessus* subsp. *massiliense* was observed and the differences in single infection were clearly differentiated from the uninfected macrophages (Figure 10). These data indicate that the immunoregulation of HCMV infection was dominantly achieved, except when other pathogens effectively infected.

HCMV promotes the *M. abscessus* subsp. *massiliense* proliferation of through the expression of cmvIL-10 and induction of host IL-10

To evaluate the effect of IL-10 on the proliferation of *M. abscessus* subsp. *massiliense*, an IL-10 receptor-neutralizing antibody (IL-10R NAb) was added to the culture of *M. abscessus* subsp. *massiliense*-infected macrophages. To neutralize the effects of endogenous human IL-10. The result confirmed that the level of growth of the bacteria was regulated by IL-10R Ab in a dose-dependent manner from HCMV-infected THP-1 macrophages (Figure 11) and PBMCs. Neutralization of IL-10R did not affect *M. abscessus* subsp. *massiliense* growth without HCMV infection under other conditions. Treatment with neutralizing antibodies against cmvIL-10 (cIL-10 NAb) or human IL-10 (IL-10 NAb) reduced bacterial growth from HCMV-infected THP-1 macrophages and PBMCs (Figure 12). HCMV further supports the concept of promoting IL-10 production in the host through the expression of cmvIL-10 and inhibiting the antibacterial activity of the host. Overall, these results indicate that HCMV regulates the immune response of macrophages to induce host IL-10 production and then co-exist with co-infecting *M. abscessus* subsp. *massiliense.*

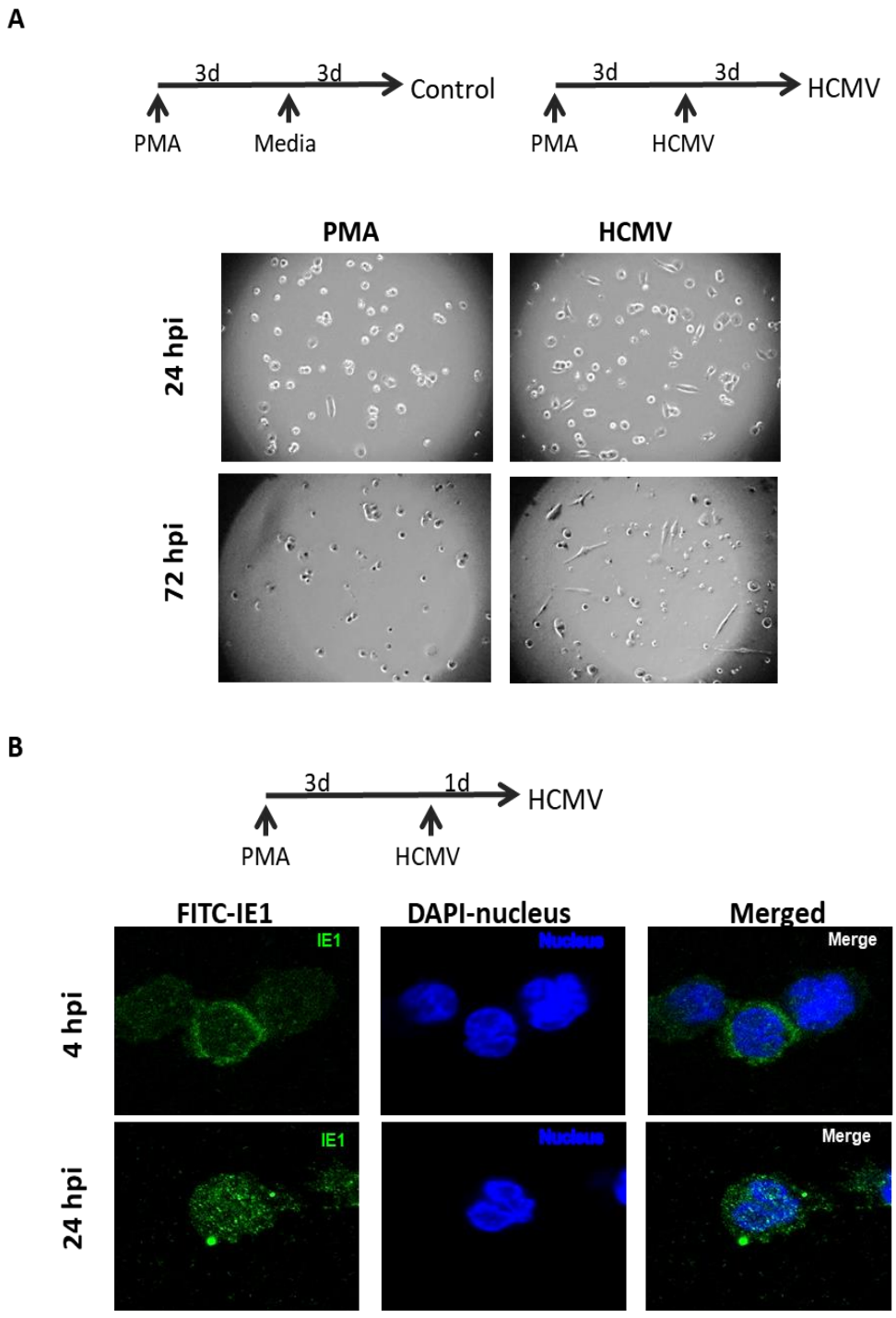

Figure 1. CPE formation and the expression of viral protein in THP-1 differentiated macrophages were confirmed using microscope.

(A) THP-1 macrophages were infected with HCMV Towne at a

MOI of 4 for 3 days, CPE was confirmed at 3 days after infection using microscope. (B) Three days after PMA treatment, HCMV was infected, IE-1 protein was stained with fluorescein isothiocyanate (FITC), nuclei were stained with DAPI, and observed with a fluorescence microscope.

Table 1. Primer set designed for HCMV *US17* detection.[83]

Name	Sequence (5'–> 3')
Forward	GCG TGC TTT TTA GCC TCT GCA
Reverse	AAA AGT TTG TGC CCC AAC GGT A
Probe	FAM–TGA TCG GCG TTA TCG CGT TCT TGA TC–TAMRA

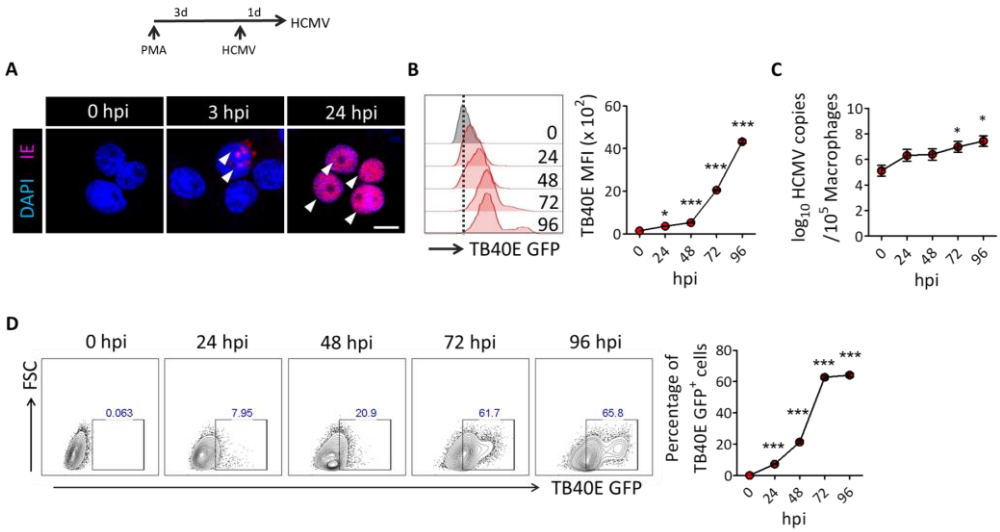

Figure 2. HCMV TB40E replicates in THP-1 differentiated macrophages.

(A) Immunofluorescence staining for the HCMV IE expression. THP-1 macrophages were infected with HCMV TB40E at a MOI of 10 for the indicated time periods. hpi, hours post infection. Scale bar = 10 μm. (B)-(D) Offset histogram for the GFP expression intensities of HCMV-TB40E-GFP infected cells obtained using flow cytometry. Quantitative graph is shown on the right side. MFI, mean fluorescence intensity. Cells infected by HCMV-TB40E-GFP obtained every 24 h. Quantitative graph is shown on the right side D. Fluorescence-activated cell sorting (FACS) graph plotting cell granularity (forward scatter [FSC]) versus GFP fluorescence intensity. Uninfected cells determined GFP background. The square marks the population considered positive. (C) HCMV *US17* copy numbers determined by qPCR. 24h post CMV infection, 1h post *M. abscessus* subsp. *massiliense* infection. All data are representative of three independent experiments. One-way ANOVA was performed. *, p<0.05, ***, p<0.001.

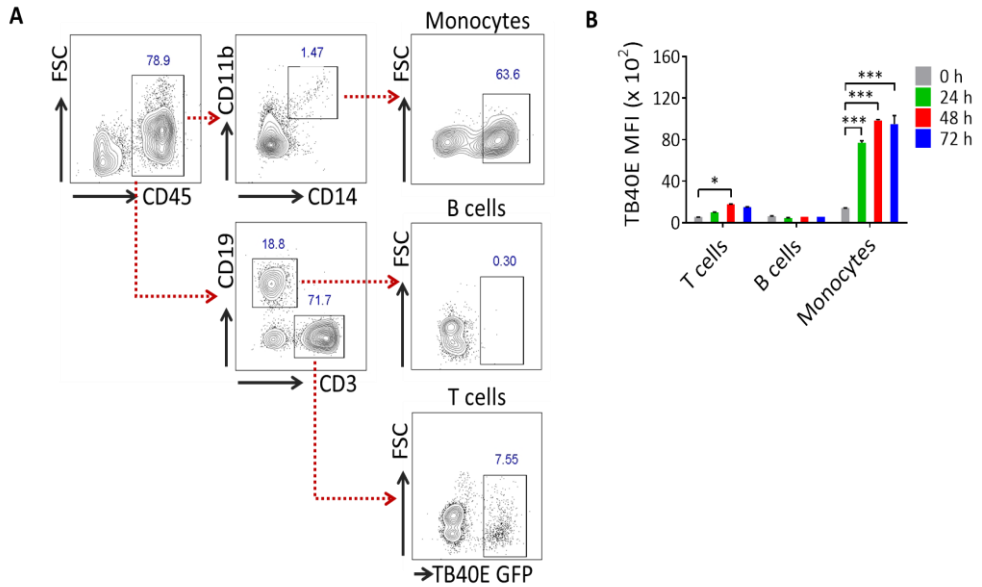

Figure 3. HCMV replicates in monocytes of human peripheral blood mononuclear cells (PBMCs).

(A) Representative contour plots for the gating strategy. PBMCs isolated from healthy volunteers were infected with HCMV TB40E strain at a MOI of 10, obtained every 24h. Staining for the expression of CD45, a common leukocyte biomarker, CD45+CD14+CD11b+ monocytes, CD45+CD3−CD19+ B cells and CD45+CD3+CD19− T cells were further analyzed for the expression of GFP at 72 h post infection. N=3 of individual cases. (B) Quantitative graph indicating the MFI of GFP expression cells assessed by flow cytometry. All data are representative of three independent experiments. P values were determined by one−way ANOVA. *, $p<0.05$, ***, $p<0.001$.

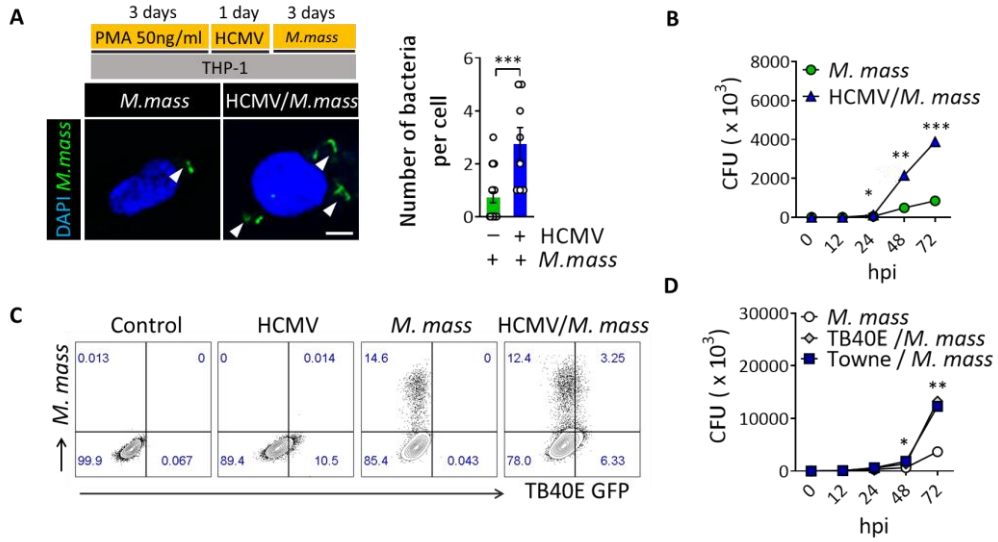

Figure 4. HCMV promotes *M. abscessus* subsp. *massiliense* proliferation in THP-1 differentiated macrophages.

(A) CFSE-labeled *M. abscessus* subsp. *massiliense* (*M. mass*, MOI = 2) was used to infect the control and HCMV TB40E-infected (MOI = 10) THP-1 cells; the cells were examined at 24 h post *M. abscessus* subsp. *massiliense* infection under a fluorescence microscope. Representative images are shown. Number of bacteria per cell is quantitated in the left graph. Scale bar = 5 μm. (B) Quantification of the CFUs of *M. abscessus* subsp. *massiliense* at the indicated time points. (C) As a result of FACS analysis, plots of single infection, and co-infection cells were analyzed. (D) Measurement of CFU of *M. abscessus* subsp. *massiliense* infected cells, at 24 h prior HCMV two strains infected. All data are representative of three independent experiments. One-way ANOVA was performed. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

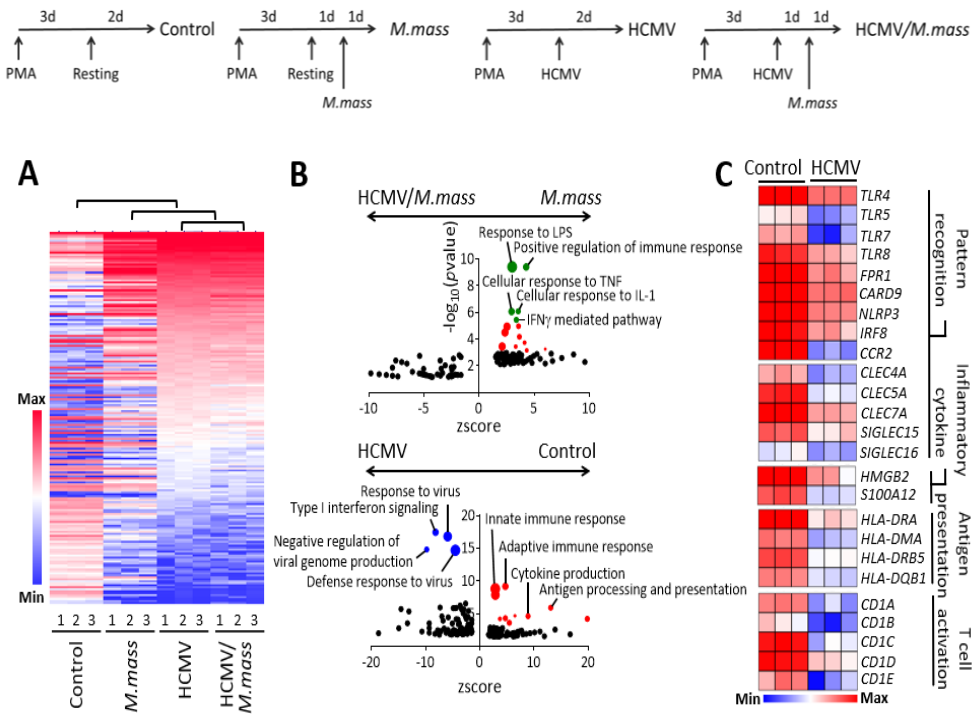

Figure 5. HCMV infection blocks pro-inflammatory and anti-bacterial immune responses in macrophages.

Schematic figure for each experimental groups for RNA sequencing; Control, *M. abscessus* subsp. *massiliense*-infected (*M. mass,* MOI = 2, 24 h), HCMV-infected (MOI = 10, 48 h), and HCMV/ *M. abscessus* subsp. *massiliense* co-infected. Triplicates per group from a single experiment were used. (A) Cellular gene expression clustering analysis, control, *M. abscessus* subsp. *massiliense*-infected, HCMV-infected, and HCMV/ *M. abscessus* subsp. *massiliense* co-infected. Red in the heatmap denotes upregulation while blue denotes downregulation. (B) Biological processes enriched in the gene ontology analysis of *M. abscessus* subsp. *massiliense*-infected versus HCMV/ *M. abscessus* subsp. *massiliense*-infected (Upper) and control versus HCMV-infected

macrophages (Bottom) are shown as GO plots. (C) Heatmaps showing differentially expressed genes associated with pattern recognition, inflammatory cytokine, antigen presentation, and T cell activation in the control and HCMV-infected cells. Warm color (red) represents an increase of gene expression while cold color (blue) determines a decrease compared to average level.

Table 2. Comparison of gene expression among the control, *M. abscessus* subsp. *massiliense* –infected (*M. mass*), HCMV−infected, and HCMV/ *M. abscessus* subsp. *massiliense* co−infected.

Top 35 scoring genes per category were ranked by *p* value.

Up regulated genes in HCMV compared to Control	Down regulated genes in HCMV compared to Control	Up regulated genes in *M.mass* compared to HCMV/*M.mass*	Down regulated genes in *M.mass* compared to HCMV/*M.mass*
IFI27	VASP	IL12B	PELI3
CCR7	PIGZ	IL23A	SECTM1
ANKRD1	RPS4X	IGFBP5	FOXC1
IDO1	AKNA	RAB27B	HDAC11
OTOF	CPE	TRPM1	SV2A
PTGES	ZNF296	DLL4	SEMA3G
RSAD2	DUSP22	C1QA	RHBDF1
AIM2	LOC101927021	CACNA2D3	SCHIP1
CA12	GTF2H2C	FGF10-AS1	EPB41L5
CXCL11	RNF19A	PTAFR	PTPN3
CCL1	CHP1	RARRES1	MMP13
CCL8	ANO6	C1QC	LOC283335
CXCL13	LOC100131564	ANO3	NUPR1
C1R	RIMBP3C	TTYH2	ABCG1
RRAD	SASH3	IL1B	ECE2
C1S	GLUD1	CASQ1	MAP2
SSTR2	FNBP1	IL1A	ZNF593
APOBEC3A	SLC22A15	LOC440896	ATP6V0E2
GFPT2	RPL34	S100A12	EPHB2
TNFAIP6	AGTPBP1	MMP1	URAHP
MAFA	RUNX1	TNFRSF18	FAM174B
SNAR-B1	POC5	LYZ	SIT1
C1QTNF1	GCNT2	PKP1	NES
SNAR-G1	CAPZB	EBI3	DLG2
COL22A1	FAM109A	ANO9	MYOT
CXCL8	MOSPD3	CCL20	PRSS16
CCND2	ADD3	C15orf48	FOSB
IFI44L	CXorf23	FCGR2C	GPRC5C
SNAR-B2	NAGA	FCGBP	SLFN13
IFIT3	FGD3	G0S2	TYRO3
OASL	SLC16A1	HLA-DQA2	LMTK3
SNAR-C4	TMEM30A	SLAMF1	USP44
SERPING1	NFATC1	FCGR2A	SEPT3
CD38	HP1BP3	AQP9	HOXA4
SLAMF7	NEGR1	DNASE1L3	B4GALT2
B4GALNT1	SNORD104	RSPO3	KHDRBS3
IFITM1	CALM1	IGDCC4	GNA11
SCG5	ABCA7	TNFRSF4	SLC38A5
PPAP2C	NCK2	CLEC4A	TFR2
ARHGAP5	IGIP	MGAT4C	ARHGAP5
TJP1	D2HGDH	CKB	CC2D1A
DUSP5	TUBA1A	CLEC4E	ZDHHC23
IFIT1	NAPRT	ADCY8	CREB5
SNAR-C3	PRPS2	OVCH1	IGFBPL1
MPDZ	PTMA	SERPINE2	FBXO16
IRG1	TPP1	CCL4L1	EGR1
IFIT2	FAM208A	BCL11A	SIX2
UCN2	GPR82	LINC00937	BEX5
HOXB7	TSPAN14	SIGLEC15	LIN7B

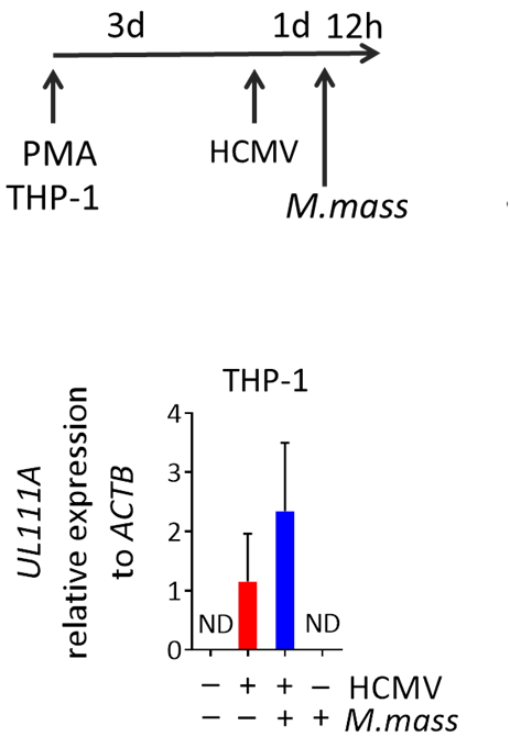

Figure 6. *UL111A* gene expression during HCMV infection.

UL111A gene relative expression was examined at 12 h HCMV (MOI = 10) post infection with *M. abscessus* subsp. *massiliense* (*M. mass*, MOI = 2) using qPCR in THP−1 differentiated macrophages. HCMV was infected (MOI = 10) at 24 h prior to *M. abscessus* subsp. *massiliense* infection. Results are means ± SEM (ND means not detected).

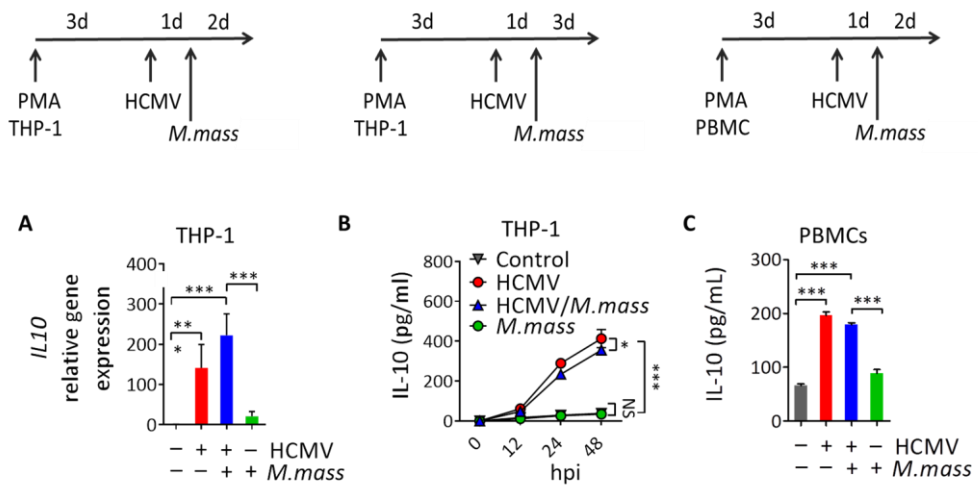

Figure 7. HCMV infection elicits host IL-10 synthesis both in THP-1 macrophages and PBMCs.

(A) Host *IL-10* gene expression was determined using qPCR in THP-1 differentiated macrophages. HCMV was infected (MOI = 10) at 24 h prior to *M. abscessus* subsp. *massiliense* infection, (*M. mass,* MOI = 2) at 48 hpi was measured using qPCR. (B) Host IL-10 in the culture supernatants of THP-1 differentiated macrophages was measured using ELISA. (C) Host IL-10 in the culture supernatants of PBMCs at 48 hpi was measured using ELISA. All data are representative of three independent experiments. One-way ANOVA or Student's t-test was performed. Results are means ± SEM, and *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$ (NS: nonsignificant).

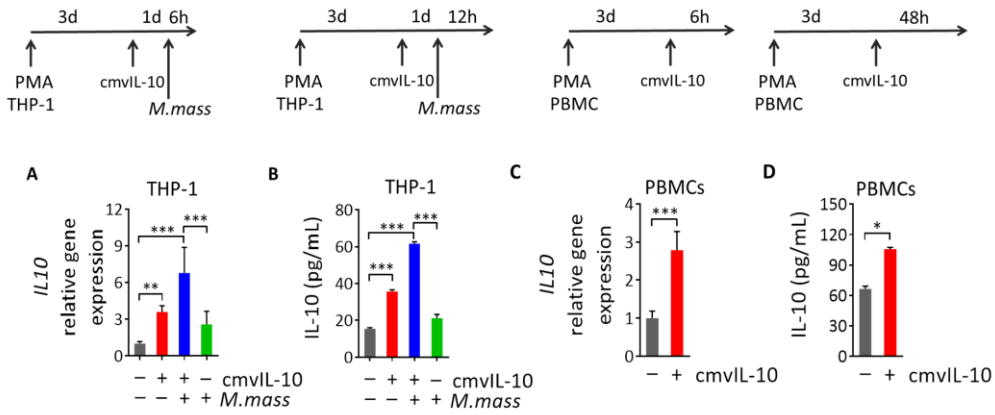

Figure 8. Viral IL-10 of HCMV induces host IL-10 synthesis in THP-1 macrophages and PBMCs.

(A) Host *IL-10* gene expression was determined in THP-1 differentiated macrophages. THP-1 macrophage treated with 200 ng/mL of cmvIL-10 for 6 h at 24 h prior to *M. abscessus* subsp. *massiliense* infection, (*M. mass,* MOI = 2). qPCR is used for the relative measurement of host *IL-10* expression. (B) Host IL-10 in the culture supernatants of THP-1 differentiated macrophages was measured using ELISA. THP-1 macrophages treated with 200 ng/mL of cmvIL-10 for 48 h. (C) For the relative measurement of host *IL-10* gene expression was determined using qPCR in PBMCs. PBMCs treated with 200 ng/mL of cmvIL-10 for 6 h. (D) Host IL-10 in the culture supernatant of PBMCs treated with 200 ng/mL of cmvIL-10 for 48 h was measured using ELISA. All data are representative of three independent experiments. ANOVA or Student's t-test was performed. *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

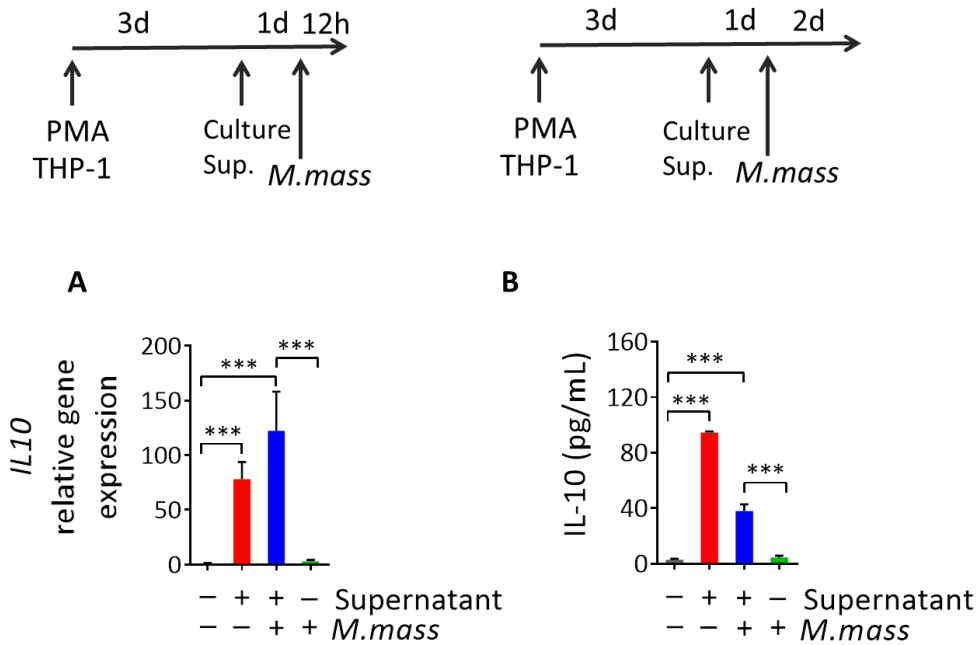

Figure 9. Culture supernatant of HCMV-infected macrophages induces human IL-10 synthesis in THP-1 macrophages.

(A) Host *IL-10* gene expression at 12 hpi was determined using qPCR in THP-1 differentiated macrophages. Culture media of differentiated THP-1 macrophages was changed with HCMV culture supernatant (MOI = 10, 48) at 24 h prior to *M. abscessus* subsp. *massiliense* infection (*M. mass,* MOI = 2). (B) IL-10 in the culture supernatant of THP-1 macrophages at 48 hpi was determined by ELISA. Culture media of differentiated THP-1 macrophages was changed with HCMV culture supernatant at 24 h prior to *M. abscessus* subsp. *massiliense* infection. All data are representative of three independent experiments. One-way ANOVA was performed. ***, $p<0.001$.

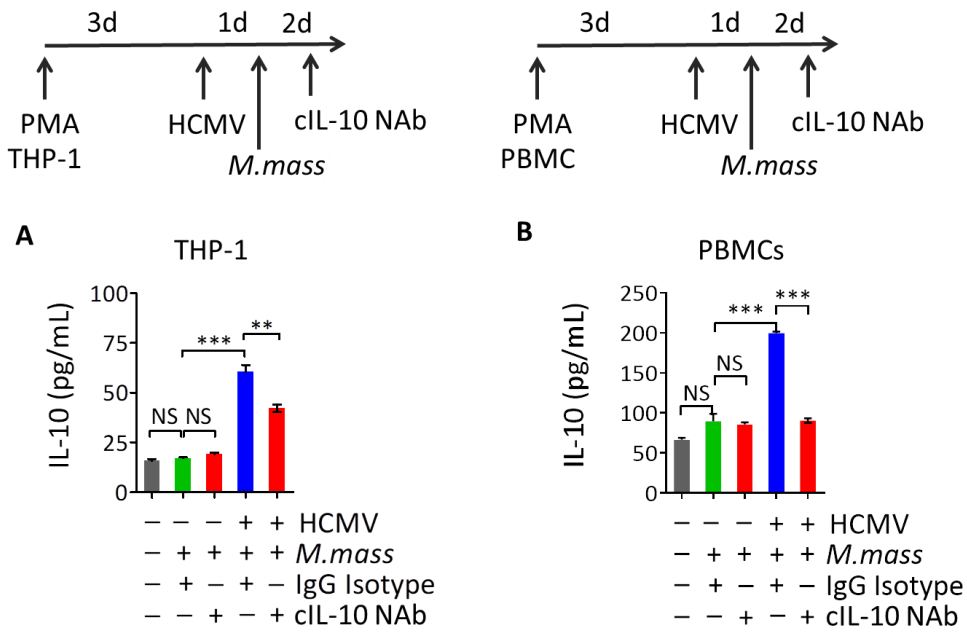

Figure 10. Host IL-10 synthesis decreases in the HCMV-infected THP-1 differentiated macrophages and PBMCs with cmvIL-10 neutralizing antibody.

IL-10 in the culture supernatant of THP-1 macrophages (A) and PBMCs (B) was determined by ELISA. HCMV was infected (MOI = 10) at 24 h prior to *M. abscessus* subsp. *massiliense* infection, (*M. mass,* MOI = 2) Neutralization antibodies for the cmvIL-10 (cIL-10 NAb, 10 mg/mL) or corresponding isotype controls were added at 3 h post HCMV infection. The results are representative of two independent experiments and represent means ± SEM. One-way ANOVA or Student's t-test was performed: NS, nonsignificant; **, $p<0.01$, ***, $p<0.001$.

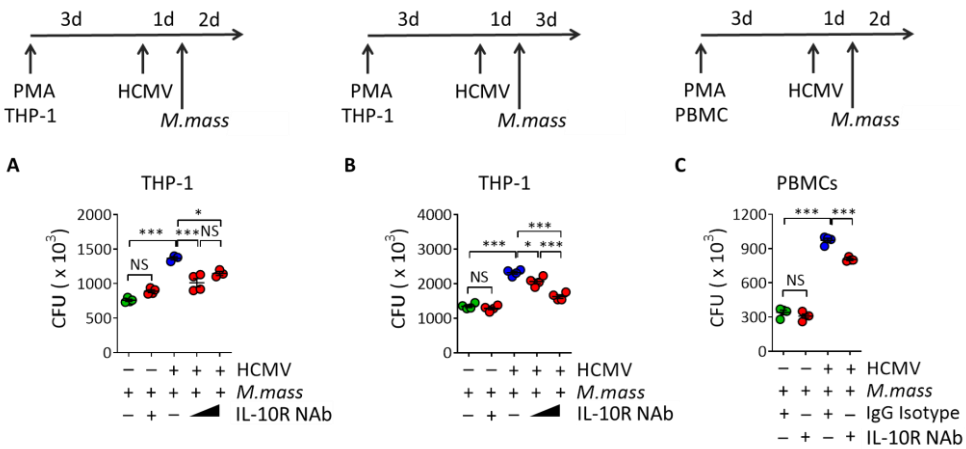

Figure 11. Bacterial burden decreases in an IL-10R antibody in the HCMV-infected THP-1 macrophages and PBMCs.

(A), (B) CFUs of *M. abscessus* subsp. *massiliense* (*M. mass*) at 48, 72 hpi. HCMV was infected (MOI = 10) at 24 h prior *M. abscessus* subsp. *massiliense* challenge in THP-1 differentiated macrophages. (MOI = 2) Neutralization antibodies for the IL-10 receptor antibody (IL-10R NAb, 1 and 10 mg/mL) were added. (C) CFUs of *M. abscessus* subsp. *massiliense* at 48 hpi in PBMCs. HCMV was infected at 24 h prior *M. abscessus* subsp. *massiliense* challenge. A 10 mg/mL of neutralization antibodies for the IL-10 receptor antibody (IL-10R NAb) was added. All data are representative of three independent experiments. One-way ANOVA was performed: NS, nonsignificant; *, $p<0.05$, ***, $p<0.001$

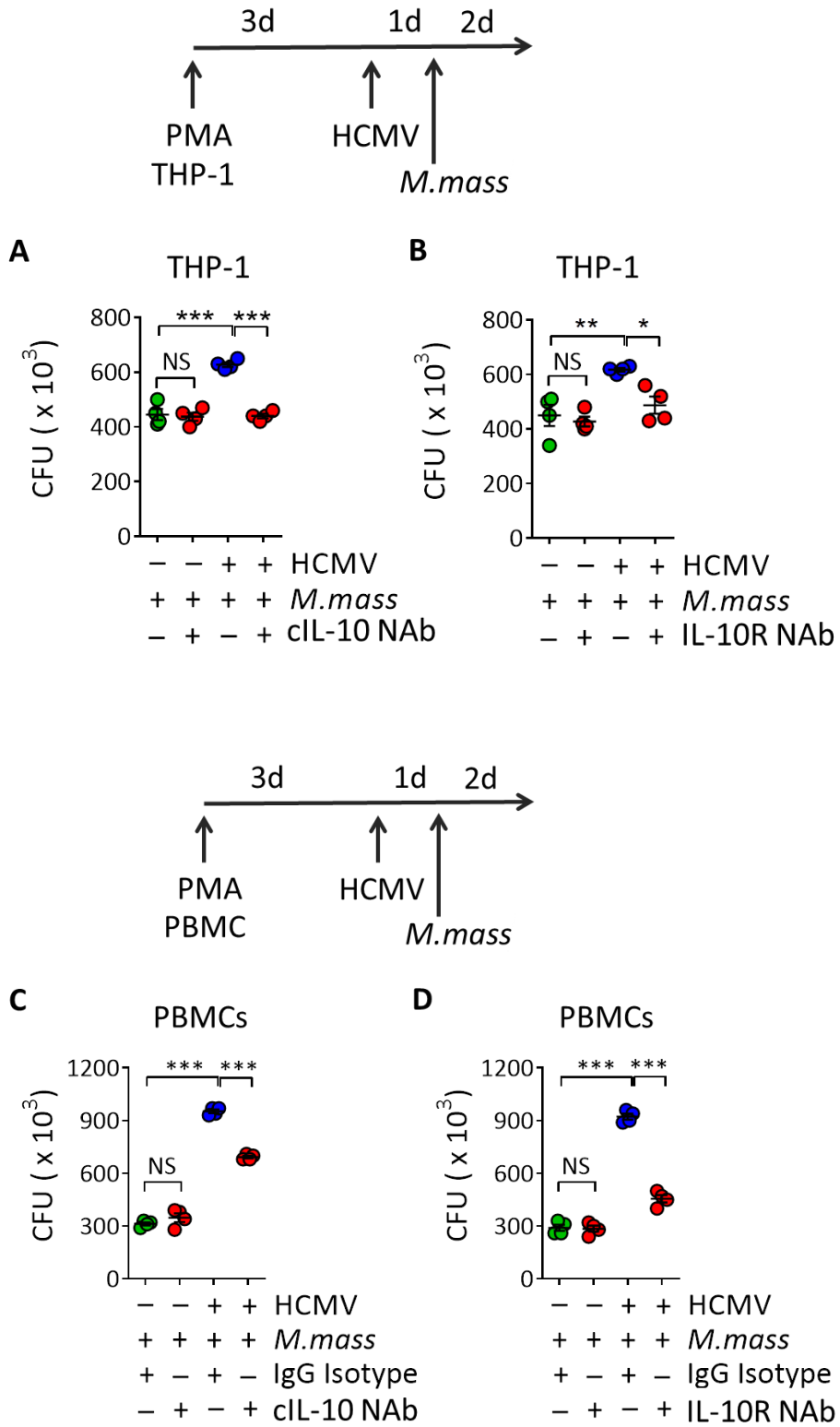

Figure 12. Bacterial burden decreases in cIL-10 or IL-10R

neutralizing antibodies in the HCMV-infected THP-1 macrophages and PBMCs.

(A)-(B)CFUs of *M. abscessus* subsp. *massiliense* (*M. mass*) at 48 hpi. HCMV was infected (MOI = 10) at 24 h prior *M. abscessus* subsp. *massiliense* challenge. (MOI = 2) Neutralization antibodies for the cmvIL-10 (cIL-10 NAb, 10 mg/mL), human IL-10 antibodies (IL-10R NAb, 10 mg/mL) or corresponding isotype controls were added at 3 h post HCMV infection. (C)-(D) CFUs of *M. abscessus* subsp. *massiliense* at 48 hpi in PBMCs. HCMV was infected at 24 h prior *M. abscessus* subsp. *massiliense* challenge. A cmvIL-10 (cIL-10 NAb), human IL-10 antibodies (IL-10 NAb) or corresponding isotype controls were added at 3 h post HCMV infection. All data are representative of three independent experiments. One-way ANOVA was performed: NS, nonsignificant; *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

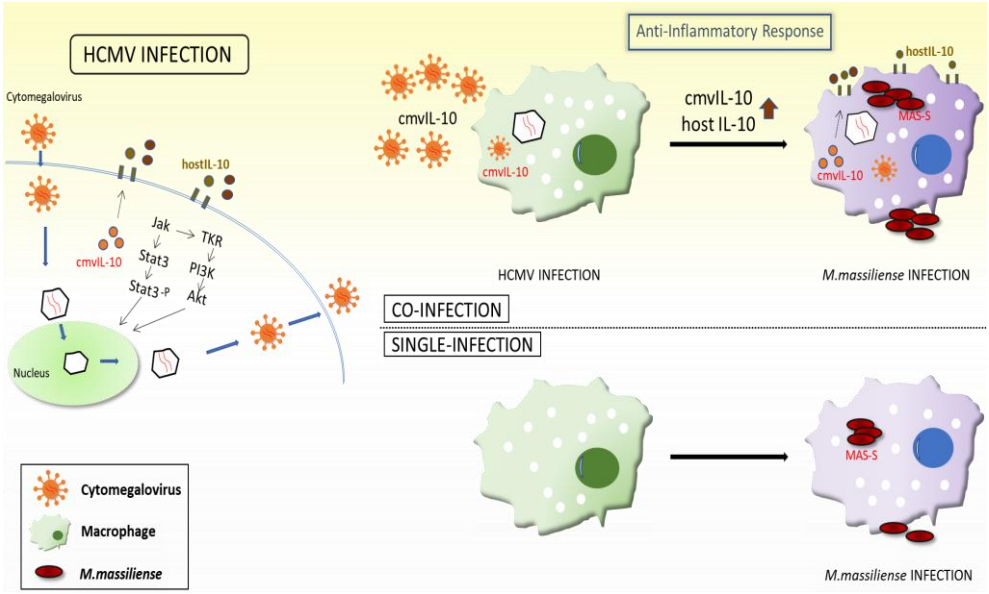

Figure 13. Schematic summary.

HCMV infection expresses cmvIL-10 and regulates the host IL-10 signaling. Inhibition of pro-inflammatory cytokines by IL-10 promotes the ability to stimulate macrophage phenotypes for anti-inflammation and to induce *M. abscessus* subsp. *massiliense* proliferation. HCMV-infected macrophages have an increased burden of *M. abscessus* subsp. *massiliense* through the regulation of IL-10 in host cells.

DISCUSSION

HCMV is a ubiquitous virus that directly or indirectly affects human diseases. The initial response of the innate immunity to a pathogen determines the outcome and direction of what caused a systemic immune response.[84]

HCMV-infected monocytes promote transendothelial migration and gather around the tissue to enter and flow through blood vessels. HCMV induces the establishment of a long-lived macrophage phenotype that can infect monocytes and cause chronic diseases. It was also confirmed in this study that HCMV can be continuously replicated in differentiated monocytes and macrophages (Figure 1, 2), and serves as an important site for organ production and excretion.

Because of the virus, the process of differentiation from monocytes to macrophages is highly likely to create an environment for viral replication and long-term virus survival.[85; 86; 87; 88]

After HCMV infection, macrophages induce a M1 macrophage-like phenotype to enable changes in adhesion, motility, and survival, and it is thought to be possible to regulate the weakening of the antiviral immune response through the upregulation of M2-related genes.[87; 88] This change in the tightrope of macrophages does not only induce polarization of HCMV, but also promotes the alteration of the already differentiated polarization profile.[89] HCMV weighs up the pros and cons of these cells to enable long-term virus survival and replication.

Virus-related pathogenesis has infection-induced inflammatory responses, including cytokine responses. Understanding the mechanism of cmv-induced cytokines may help in the clinical

management of the disease. As a feedback regulator, virus-induced cytokines activate the immune response that occurs and regulates the adaptive immune response. Many pathogens, including viruses, regulate cytokine expression to overcome antiviral responses.[90] Cytokines can also promote CMV reactivation and subsequent replication,[54] indirectly improving tissue damage.

This study was to observe the change in the host signal during bacterial co-infection through the regulation of the immune response after viral infection. In addition to the general IFN response, the study confirmed that HCMV enhanced the production of IL-10 by the host macrophages. This phenomenon potentially altered the antibacterial defense mechanism. IL-10 blocks phagosome maturation[91] and suppresses the production of pro-inflammatory cytokines,[92; 93] which was detected in the results of this study and increased by HCMV infection.

The immunosuppressive function of cmvIL-10 is very important in many pathogens, including viruses. Regulation of cmvIL-10 expression can modulate the immune response of host cells, overcome the antiviral response, and maintain a latent infection state.[94] In addition, viral IL-10 reduces the production of TNF-α and IL-1β through the upregulation of HO-1.[95] However, this situation is established when the viral IL-10 remains more dominant. In HCV and HIV infection as an example of such persistent infection, it was confirmed that host IL-10 was expressed at a high level.[96; 97; 98]

The cmvIL-10 protein is similar to 27% amino acid identity with host IL-10 but retains its ability to bind to the host IL-10 receptor.[65] cmvIL-10 increases the secretion of host IL-10 from monocytes, macrophages and dendritic cells, thus amplifying

IL-10-mediated immune suppression and creating an environment for chronic infection.[61]

As described previously, depending on the current geographical location and socioeconomic status, there is a high HCMV infection rate worldwide,[99; 100] and HCMV infection has the potential to affect infections with new problems. According to a recent report, HCMV infection is known to have a mechanically relevant role in the risk of tuberculosis by facilitating its progression through potential tuberculosis infections.[101; 102; 103]

Comparing other examples of pathogens that single infect and co-infect as persistent or latent infections, the antigenic escape effect of HCMV can be confirmed. Tuberculosis or HIV infection generally creates an immune environment that increases inflammation,[104; 105] enabling the reactivation of HCMV.[106] CMV can cause vision loss, neurological, and gastrointestinal diseases in HIV co-infection conditions.[79; 107; 108]

Chronic or persistent HCMV-infected patients are simultaneously infected with pulmonary tuberculosis and take long-term treatments with anti-NTM drugs. It has been possible to indirectly predict that clinical disease progression is promoted due to abnormal immune responses.

In this study, used two HCMV strains, a laboratory strain Towne and TB40E with the GFP. For the Towne strain, one should be careful in interpreting the results from tropism and pathogenesis studies to be considered representative of causative factors of clinical disease.[109] TB40E strain retains a broad range of cell tropism including fibroblasts and endothelial cells. The two strains grew differently depending on the cell type,[110] but did not show any difference in THP-1 macrophage.

There were several limitations of this study. First the number of CMV seropositive donors was low. Validation with a larger population is needed to analyze the socio-economic location-based conditions between CMV infection and tuberculosis risk. In addition, no data were obtained from the virus *UL111A* gene, which has been deleted. Even so, the study results are important because they provide a predictable basis.

According to a recent report by Cobelens et al, HCMV infection is known to have an epidemiological role in the influence of tuberculosis by promoting disease-induced progression in latent tuberculosis infection.[77] This makes it possible to prevent the macrophages from being activated and prevent the macrophages from secreting reactive nitrogen species.[111] The results of study indicate that the synthesis of host IL-10 by HCMV infection regulates the ability to induce macrophage phenotypes for anti-inflammation and to promote *M. abscessus* subsp. *massiliense* smooth strain proliferation (Figure 13). This study found that HCMV-infected macrophages have an increased burden of *M. abscessus* subsp. *massiliense* smooth strain through the regulation of IL-10 in host cells.

REFERENCES

[1] R.J. Whitley, and B. Roizman, Herpes simplex virus infections. Lancet 357 (2001) 1513-8.

[2] S. Varani, and M.P. Landini, Cytomegalovirus-induced immunopathology and its clinical consequences. Herpesviridae 2 (2011) 6.

[3] V. Schottstedt, J. Blumel, R. Burger, C. Drosten, A. Groner, L. Gurtler, M. Heiden, M. Hildebrandt, B. Jansen, T. Montag-Lessing, R. Offergeld, G. Pauli, R. Seitz, U. Schlenkrich, J. Strobel, H. Willkommen, and C.H. von Konig, Human Cytomegalovirus (HCMV) - Revised. Transfus Med Hemother 37 (2010) 365-75.

[4] in: K.R. Stratton, J.S. Durch, and R.S. Lawrence, (Eds.), Vaccines for the 21st Century: A Tool for Decisionmaking, Washington (DC), (2000) 165-72.

[5] E. Murphy, and T. Shenk, Human cytomegalovirus genome. Curr Top Microbiol Immunol 325 (2008) 1-19.

[6] M.A. Jarvis, and J.A. Nelson, Human cytomegalovirus persistence and latency in endothelial cells and macrophages. Curr Opin Microbiol 5 (2002) 403-7.

[7] C. Chatzakis, Y. Ville, G. Makrydimas, K. Dinas, A. Zavlanos, and A. Sotiriadis, Timing of primary maternal cytomegalovirus infection and rates of vertical transmission and fetal consequences. Am J Obstet Gynecol 223 (2020) 870-83 e11.

[8] A.W. Sylwester, B.L. Mitchell, J.B. Edgar, C. Taormina, C. Pelte, F. Ruchti, P.R. Sleath, K.H. Grabstein, N.A. Hosken, F. Kern, J.A. Nelson, and L.J. Picker, Broadly targeted human cytomegalovirus-specific CD4+ and CD8+ T cells dominate the memory compartments of exposed subjects. J Exp Med 202 (2005) 673-85.

[9] R.E. James, J. Hillis, I. Adorjan, B. Gration, M.V. Mundim, A.J. Iqbal, M.M. Majumdar, R.L. Yates, M.M. Richards, G.E. Goings, G.C. DeLuca, D.R. Greaves, S.D. Miller, and F.G. Szele, Loss of galectin-3 decreases the number of immune cells in the subventricular zone and restores proliferation in a viral model of multiple sclerosis. Glia 64 (2016) 105-21.

[10] M.J. Adams, and E.B. Carstens, Changes to the Statutes and Subcommittees of the International Committee on Taxonomy of Viruses (2014). Archives of Virology 159 (2014) 2829-30.

[11] J.L. Stern, and B. Slobedman, Human cytomegalovirus latent infection of myeloid cells directs monocyte migration by up-regulating monocyte chemotactic protein-1. J Immunol 180 (2008) 6577-85.

[12] C. Sinzger, and G. Jahn, Human cytomegalovirus cell tropism and pathogenesis. Intervirology 39 (1996) 302-19.

[13] S. Manicklal, V.C. Emery, T. Lazzarotto, S.B. Boppana, and R.K. Gupta, The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev 26 (2013) 86-102.

[14] R.S. Gupta, B. Lo, and J. Son, Phylogenomics and Comparative Genomic Studies Robustly Support Division of the Genus *Mycobacterium* into an Emended Genus *Mycobacterium* and Four Novel Genera. Front Microbiol 9 (2018) 67.

[15] M. Moore, and J.B. Frerichs, An unusual acid-fast infection of the knee with subcutaneous, abscess-like lesions of the gluteal region; report of a case with a study of the organism, *Mycobacterium abscessus*, n. sp. J Invest Dermatol 20 (1953) 133-69.

[16] K. Ruger, A. Hampel, S. Billig, N. Rucker, S. Suerbaum, and F.C. Bange, Characterization of rough and smooth morphotypes of

Mycobacterium abscessus isolates from clinical specimens. J Clin Microbiol 52 (2014) 244-50.

[17] B.A. Kendall, and K.L. Winthrop, Update on the epidemiology of pulmonary nontuberculous mycobacterial infections. Semin Respir Crit Care Med 34 (2013) 87-94.

[18] D.R. Prevots, and T.K. Marras, Epidemiology of human pulmonary infection with nontuberculous mycobacteria: a review. Clin Chest Med 36 (2015) 13-34.

[19] A.D.S. Peixoto, L.M.L. Montenegro, A.S. Lima, F.L. Melo, W.L. Barbosa Junior, M.M.C. Neves, J.P. Ramos, H.C. Schindler, and Z.M. Medeiros, Identification of nontuberculous mycobacteria species by multiplex real-time PCR with high-resolution melting. Rev Soc Bras Med Trop 53 (2020) e20200211.

[20] E. Tortoli, T.A. Kohl, B.A. Brown-Elliott, A. Trovato, S.C. Leao, M.J. Garcia, S. Vasireddy, C.Y. Turenne, D.E. Griffith, J.V. Philley, R. Baldan, S. Campana, L. Cariani, C. Colombo, G. Taccetti, A. Teri, S. Niemann, R.J. Wallace, Jr., and D.M. Cirillo, Emended description of *Mycobacterium abscessus*, *Mycobacterium abscessus* subsp. *abscessus* and *Mycobacterium abscessus* subsp. *bolletii* and designation of *Mycobacterium abscessus* subsp. *massiliense* comb. nov. Int J Syst Evol Microbiol 66 (2016) 4471-9.

[21] T. Adekambi, M. Sassi, J. van Ingen, and M. Drancourt, Reinstating *Mycobacterium massiliense* and *Mycobacterium bolletii* as species of the *Mycobacterium abscessus* complex. Int J Syst Evol Microbiol 67 (2017) 2726-30.

[22] B.J. Kim, B.R. Kim, S.Y. Lee, Y.H. Kook, and B.J. Kim, Rough colony morphology of *Mycobacterium massiliense* Type II genotype is due to the deletion of glycopeptidolipid locus within its genome. BMC Genomics 14 (2013) 890.

[23] A. Pawlik, G. Garnier, M. Orgeur, P. Tong, A. Lohan, F. Le Chevalier, G. Sapriel, A.L. Roux, K. Conlon, N. Honore, M.A. Dillies, L. Ma, C. Bouchier, J.Y. Coppee, J.L. Gaillard, S.V. Gordon, B. Loftus, R. Brosch, and J.L. Herrmann, Identification and characterization of the genetic changes responsible for the characteristic smooth-to-rough morphotype alterations of clinically persistent *Mycobacterium abscessus*. Mol Microbiol 90 (2013) 612-29.

[24] S.T. Howard, E. Rhoades, J. Recht, X. Pang, A. Alsup, R. Kolter, C.R. Lyons, and T.F. Byrd, Spontaneous reversion of *Mycobacterium abscessus* from a smooth to a rough morphotype is associated with reduced expression of glycopeptidolipid and reacquisition of an invasive phenotype. Microbiology (Reading) 152 (2006) 1581-90.

[25] R. Mukherjee, and D. Chatterji, Glycopeptidolipids: immuno-modulators in greasy mycobacterial cell envelope. IUBMB Life 64 (2012) 215-25.

[26] E. Catherinot, J. Clarissou, G. Etienne, F. Ripoll, J.F. Emile, M. Daffe, C. Perronne, C. Soudais, J.L. Gaillard, and M. Rottman, Hypervirulence of a rough variant of the *Mycobacterium abscessus* type strain. Infect Immun 75 (2007) 1055-8.

[27] J. Whang, Y.W. Back, K.I. Lee, N. Fujiwara, S. Paik, C.H. Choi, J.K. Park, and H.J. Kim, *Mycobacterium abscessus* glycopeptidolipids inhibit macrophage apoptosis and bacterial spreading by targeting mitochondrial cyclophilin D. Cell Death Dis 8 (2017) e3012.

[28] P. Chakraborty, and A. Kumar, The extracellular matrix of mycobacterial biofilms: could we shorten the treatment of mycobacterial infections? Microb Cell 6 (2019) 105-22.

[29] B.J. Kim, T.S. Shim, S.Y. Yi, H.C. Kim, B.R. Kim, S.Y. Lee, Y.H.

Kook, and B.J. Kim, *Mycobacterium massiliense* Type II genotype leads to higher level of colony forming units and TNF-alpha secretion from human monocytes than Type I genotype. APMIS 123 (2015) 895-902.
[30] M.R. Lee, W.H. Sheng, C.C. Hung, C.J. Yu, L.N. Lee, and P.R. Hsueh, *Mycobacterium abscessus* Complex Infections in Humans. Emerg Infect Dis 21 (2015) 1638-46.
[31] D.E. Griffith, T. Aksamit, B.A. Brown-Elliott, A. Catanzaro, C. Daley, F. Gordin, S.M. Holland, R. Horsburgh, G. Huitt, M.F. Iademarco, M. Iseman, K. Olivier, S. Ruoss, C.F. von Reyn, R.J. Wallace, Jr., K. Winthrop, A.T.S.M.D. Subcommittee, S. American Thoracic, and A. Infectious Disease Society of, An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. Am J Respir Crit Care Med 175 (2007) 367-416.
[32] C.S. Haworth, J. Banks, T. Capstick, A.J. Fisher, T. Gorsuch, I.F. Laurenson, A. Leitch, M.R. Loebinger, H.J. Milburn, M. Nightingale, P. Ormerod, D. Shingadia, D. Smith, N. Whitehead, R. Wilson, and R.A. Floto, British Thoracic Society guidelines for the management of non-tuberculous mycobacterial pulmonary disease (NTM-PD). Thorax 72 (2017) ii1-64.
[33] R. Diel, F. Ringshausen, E. Richter, L. Welker, J. Schmitz, and A. Nienhaus, Microbiological and Clinical Outcomes of Treating Non-*Mycobacterium Avium* Complex Nontuberculous Mycobacterial Pulmonary Disease: A Systematic Review and Meta-Analysis. Chest 152 (2017) 120-42.
[34] J.G. Pasipanodya, D. Ogbonna, B.E. Ferro, G. Magombedze, S. Srivastava, D. Deshpande, and T. Gumbo, Systematic Review and Meta-analyses of the Effect of Chemotherapy on Pulmonary

Mycobacterium abscessus Outcomes and Disease Recurrence. Antimicrob Agents Chemother 61 (2017).

[35] L.T. Angenent, S.T. Kelley, A. St Amand, N.R. Pace, and M.T. Hernandez, Molecular identification of potential pathogens in water and air of a hospital therapy pool. Proc Natl Acad Sci U S A 102 (2005) 4860-5.

[36] J.O. Falkinham, 3rd, M.D. Iseman, P. de Haas, and D. van Soolingen, *Mycobacterium avium* in a shower linked to pulmonary disease. J Water Health 6 (2008) 209-13.

[37] D.E. Griffith, W.M. Girard, and R.J. Wallace, Jr., Clinical features of pulmonary disease caused by rapidly growing mycobacteria. An analysis of 154 patients. Am Rev Respir Dis 147 (1993) 1271-8.

[38] W.J. Koh, K. Jeon, N.Y. Lee, B.J. Kim, Y.H. Kook, S.H. Lee, Y.K. Park, C.K. Kim, S.J. Shin, G.A. Huitt, C.L. Daley, and O.J. Kwon, Clinical significance of differentiation of *Mycobacterium massiliense* from *Mycobacterium abscessus*. Am J Respir Crit Care Med 183 (2011) 405-10.

[39] F.M. Collins, AIDS-related mycobacterial disease. Springer Semin Immunopathol 10 (1988) 375-91.

[40] F.M. Collins, Mycobacterial disease, immunosuppression, and acquired immunodeficiency syndrome. Clin Microbiol Rev 2 (1989) 360-77.

[41] Y. Choi, K.S. Lee, S.K. Kim, and W.J. Koh, Unilateral Lung Involvement of Nodular Bronchiectatic *Mycobacterium Avium* Complex Pulmonary Diseases: Proportion and Evolution on Serial CT Studies. AJR Am J Roentgenol (2019) 1-8.

[42] J. van Ingen, M.J. Boeree, D. van Soolingen, and J.W. Mouton, Resistance mechanisms and drug susceptibility testing of

nontuberculous mycobacteria. Drug Resist Updat 15 (2012) 149-61.

[43] K.A. Nash, B.A. Brown-Elliott, and R.J. Wallace, Jr., A novel gene, erm(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. Antimicrob Agents Chemother 53 (2009) 1367-76.

[44] H. Choi, B.W. Jhun, S.Y. Kim, D.H. Kim, H. Lee, K. Jeon, O.J. Kwon, H.J. Huh, C.S. Ki, N.Y. Lee, S.J. Shin, C.L. Daley, and W.J. Koh, Treatment outcomes of macrolide-susceptible *Mycobacterium abscessus* lung disease. Diagn Microbiol Infect Dis 90 (2018) 293-5.

[45] S.E. Strollo, J. Adjemian, M.K. Adjemian, and D.R. Prevots, The Burden of Pulmonary Nontuberculous Mycobacterial Disease in the United States. Ann Am Thorac Soc 12 (2015) 1458-64.

[46] M.L. Wu, D.B. Aziz, V. Dartois, and T. Dick, NTM drug discovery: status, gaps and the way forward. Drug Discov Today 23 (2018) 1502-19.

[47] H.Y. Kim, Y.J. Yun, C.G. Park, D.H. Lee, Y.K. Cho, B.J. Park, S.I. Joo, E.C. Kim, Y.J. Hur, B.J. Kim, and Y.H. Kook, Outbreak of *Mycobacterium massiliense* infection associated with intramuscular injections. J Clin Microbiol 45 (2007) 3127-30.

[48] S.C. Parrish, J. Myers, and A. Lazarus, Nontuberculous mycobacterial pulmonary infections in Non-HIV patients. Postgrad Med 120 (2008) 78-86.

[49] D. Jeon, Infection Source and Epidemiology of Nontuberculous Mycobacterial Lung Disease. Tuberc Respir Dis (Seoul) 82 (2019) 94-101.

[50] H.J. Yoon, H.Y. Choi, and M. Ki, Nontuberculosis mycobacterial

infections at a specialized tuberculosis treatment centre in the Republic of Korea. BMC Infect Dis 17 (2017) 432.

[51] H. Lee, W. Myung, W.J. Koh, S.M. Moon, and B.W. Jhun, Epidemiology of Nontuberculous Mycobacterial Infection, South Korea, 2007-2016. Emerg Infect Dis 25 (2019) 569-72.

[52] H.J. Ryu, W.J. Kim, C.H. Oh, and H.J. Song, Iatrogenic *Mycobacterium abscessus* infection associated with acupuncture: clinical manifestations and its treatment. Int J Dermatol 44 (2005) 846-50.

[53] W.J. Koh, O.J. Kwon, K. Jeon, T.S. Kim, K.S. Lee, Y.K. Park, and G.H. Bai, Clinical significance of nontuberculous mycobacteria isolated from respiratory specimens in Korea. Chest 129 (2006) 341-8.

[54] W.L. Chang, and P.A. Barry, Attenuation of innate immunity by cytomegalovirus IL-10 establishes a long-term deficit of adaptive antiviral immunity. Proc Natl Acad Sci U S A 107 (2010) 22647-52.

[55] M.T. Barel, G.C. Hassink, S. van Voorden, and E.J. Wiertz, Human cytomegalovirus-encoded US2 and US11 target unassembled MHC class I heavy chains for degradation. Mol Immunol 43 (2006) 1258-66.

[56] B.P. McSharry, S. Avdic, and B. Slobedman, Human cytomegalovirus encoded homologs of cytokines, chemokines and their receptors: roles in immunomodulation. Viruses 4 (2012) 2448-70.

[57] A. Luganini, M.E. Terlizzi, and G. Gribaudo, Bioactive Molecules Released From Cells Infected with the Human Cytomegalovirus. Front Microbiol 7 (2016) 715.

[58] E. Van Damme, and M. Van Loock, Functional annotation of human cytomegalovirus gene products: an update. Front Microbiol 5

(2014) 218.
[59] A.L. McCormick, L. Roback, D. Livingston-Rosanoff, and C. St Clair, The human cytomegalovirus UL36 gene controls caspase-dependent and -independent cell death programs activated by infection of monocytes differentiating to macrophages. J Virol 84 (2010) 5108-23.
[60] P. Bozidis, C.D. Williamson, D.S. Wong, and A.M. Colberg-Poley, Trafficking of UL37 proteins into mitochondrion-associated membranes during permissive human cytomegalovirus infection. J Virol 84 (2010) 7898-903.
[61] S. Avdic, B.P. McSharry, M. Steain, E. Poole, J. Sinclair, A. Abendroth, and B. Slobedman, Human Cytomegalovirus-Encoded Human Interleukin-10 (IL-10) Homolog Amplifies Its Immunomodulatory Potential by Upregulating Human IL-10 in Monocytes. J Virol 90 (2016) 3819-27.
[62] G. Westall, T. Kotsimbos, and A. Brooks, CMV-specific CD8 T-cell dynamics in the blood and the lung allograft reflect viral reactivation following lung transplantation. Am J Transplant 6 (2006) 577-84.
[63] W. Ouyang, S. Rutz, N.K. Crellin, P.A. Valdez, and S.G. Hymowitz, Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29 (2011) 71-109.
[64] J.M. Rojas, M. Avia, V. Martin, and N. Sevilla, IL-10: A Multifunctional Cytokine in Viral Infections. J Immunol Res 2017 (2017) 6104054.
[65] S.V. Kotenko, S. Saccani, L.S. Izotova, O.V. Mirochnitchenko, and S. Pestka, Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). Proc Natl Acad Sci U S A 97 (2000)

1695-700.
[66] K.M. Lockridge, S.S. Zhou, R.H. Kravitz, J.L. Johnson, E.T. Sawai, E.L. Blewett, and P.A. Barry, Primate cytomegaloviruses encode and express an IL-10-like protein. Virology 268 (2000) 272-80.
[67] C. Jenkins, W. Garcia, A. Abendroth, and B. Slobedman, Expression of a human cytomegalovirus latency-associated homolog of interleukin-10 during the productive phase of infection. Virology 370 (2008) 285-94.
[68] A. Boonstra, R. Rajsbaum, M. Holman, R. Marques, C. Asselin-Paturel, J.P. Pereira, E.E. Bates, S. Akira, P. Vieira, Y.J. Liu, G. Trinchieri, and A. O'Garra, Macrophages and myeloid dendritic cells, but not plasmacytoid dendritic cells, produce IL-10 in response to MyD88- and TRIF-dependent TLR signals, and TLR-independent signals. J Immunol 177 (2006) 7551-8.
[69] R. Sabat, IL-10 family of cytokines. Cytokine Growth Factor Rev 21 (2010) 315-24.
[70] M.C. Cheeran, S. Hu, W.S. Sheng, P.K. Peterson, and J.R. Lokensgard, CXCL10 production from cytomegalovirus-stimulated microglia is regulated by both human and viral interleukin-10. J Virol 77 (2003) 4502-15.
[71] T.K. Marras, D. Mendelson, A. Marchand-Austin, K. May, and F.B. Jamieson, Pulmonary nontuberculous mycobacterial disease, Ontario, Canada, 1998-2010. Emerg Infect Dis 19 (2013) 1889-91.
[72] J. Beswick, E. Shin, F.V. Michelis, S. Thyagu, A. Viswabandya, J.H. Lipton, H. Messner, T.K. Marras, and D.D.H. Kim, Incidence and Risk Factors for Nontuberculous Mycobacterial Infection after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant 24 (2018) 366-72.

[73] K.W. Moore, R. de Waal Malefyt, R.L. Coffman, and A. O'Garra, Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 19 (2001) 683-765.
[74] P.J. Murray, Understanding and exploiting the endogenous interleukin-10/STAT3-mediated anti-inflammatory response. Curr Opin Pharmacol 6 (2006) 379-86.
[75] V. Sanchez, J.J. Dong, J. Battley, K.N. Jackson, and B.C. Dykes, Human cytomegalovirus infection of THP-1 derived macrophages reveals strain-specific regulation of actin dynamics. Virology 433 (2012) 64-72.
[76] L. Stockdale, S. Nash, A. Nalwoga, H. Painter, G. Asiki, H. Fletcher, and R. Newton, Human cytomegalovirus epidemiology and relationship to tuberculosis and cardiovascular disease risk factors in a rural Ugandan cohort. PLoS One 13 (2018) e0192086.
[77] F. Cobelens, N. Nagelkerke, and H. Fletcher, The convergent epidemiology of tuberculosis and human cytomegalovirus infection. F1000Res 7 (2018) 280.
[78] J.H. Kim, J. Kim, J. Roh, C.S. Park, J.Y. Seoh, and E.S. Hwang, Reactive oxygen species-induced parthanatos of immunocytes by human cytomegalovirus-associated substance. Microbiol Immunol 62 (2018) 229-42.
[79] C. Sinzger, K. Schmidt, J. Knapp, M. Kahl, R. Beck, J. Waldman, H. Hebart, H. Einsele, and G. Jahn, Modification of human cytomegalovirus tropism through propagation in vitro is associated with changes in the viral genome. J Gen Virol 80 (Pt 11) (1999) 2867-77.
[80] E.S. Hwang, Z. Zhang, H. Cai, D.Y. Huang, S.M. Huong, C.Y. Cha, and E.S. Huang, Human cytomegalovirus IE1-72 protein interacts with p53 and inhibits p53-dependent transactivation by a

mechanism different from that of IE2-86 protein. J Virol 83 (2009) 12388-98.
[81] B. Langmead, and S.L. Salzberg, Fast gapped-read alignment with Bowtie 2. Nat Methods 9 (2012) 357-9.
[82] R.C. Gentleman, V.J. Carey, D.M. Bates, B. Bolstad, M. Dettling, S. Dudoit, B. Ellis, L. Gautier, Y. Ge, J. Gentry, K. Hornik, T. Hothorn, W. Huber, S. Iacus, R. Irizarry, F. Leisch, C. Li, M. Maechler, A.J. Rossini, G. Sawitzki, C. Smith, G. Smyth, L. Tierney, J.Y. Yang, and J. Zhang, Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5 (2004) R80.
[83] U. Machida, M. Kami, T. Fukui, Y. Kazuyama, M. Kinoshita, Y. Tanaka, Y. Kanda, S. Ogawa, H. Honda, S. Chiba, K. Mitani, Y. Muto, K. Osumi, S. Kimura, and H. Hirai, Real-time automated PCR for early diagnosis and monitoring of cytomegalovirus infection after bone marrow transplantation. J Clin Microbiol 38 (2000) 2536-42.
[84] S.H.E. Kaufmann, and A. Dorhoi, Molecular Determinants in Phagocyte-Bacteria Interactions. Immunity 44 (2016) 476-91.
[85] M.S. Smith, G.L. Bentz, J.S. Alexander, and A.D. Yurochko, Human cytomegalovirus induces monocyte differentiation and migration as a strategy for dissemination and persistence. J Virol 78 (2004) 4444-53.
[86] G. Chan, M.T. Nogalski, and A.D. Yurochko, Human cytomegalovirus stimulates monocyte-to-macrophage differentiation via the temporal regulation of caspase 3. J Virol 86 (2012) 10714-23.
[87] G. Chan, E.R. Bivins-Smith, M.S. Smith, P.M. Smith, and A.D. Yurochko, Transcriptome analysis reveals human cytomegalovirus reprograms monocyte differentiation toward an M1 macrophage. J

Immunol 181 (2008) 698-711.
[88] G. Chan, E.R. Bivins-Smith, M.S. Smith, and A.D. Yurochko, NF-kappaB and phosphatidylinositol 3-kinase activity mediates the HCMV-induced atypical M1/M2 polarization of monocytes. Virus Res 144 (2009) 329-33.
[89] E.V. Stevenson, D. Collins-McMillen, J.H. Kim, S.J. Cieply, G.L. Bentz, and A.D. Yurochko, HCMV reprogramming of infected monocyte survival and differentiation: a Goldilocks phenomenon. Viruses 6 (2014) 782-807.
[90] H. Nair, W.A. Brooks, M. Katz, A. Roca, J.A. Berkley, S.A. Madhi, J.M. Simmerman, A. Gordon, M. Sato, S. Howie, A. Krishnan, M. Ope, K.A. Lindblade, P. Carosone-Link, M. Lucero, W. Ochieng, L. Kamimoto, E. Dueger, N. Bhat, S. Vong, E. Theodoratou, M. Chittaganpitch, O. Chimah, A. Balmaseda, P. Buchy, E. Harris, V. Evans, M. Katayose, B. Gaur, C. O'Callaghan-Gordo, D. Goswami, W. Arvelo, M. Venter, T. Briese, R. Tokarz, M.A. Widdowson, A.W. Mounts, R.F. Breiman, D.R. Feikin, K.P. Klugman, S.J. Olsen, B.D. Gessner, P.F. Wright, I. Rudan, S. Broor, E.A. Simoes, and H. Campbell, Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. Lancet 378 (2011) 1917-30.
[91] S. O'Leary, M.P. O'Sullivan, and J. Keane, IL-10 blocks phagosome maturation in *Mycobacterium Tuberculosis*-infected human macrophages. Am J Respir Cell Mol Biol 45 (2011) 172-80.
[92] M. Saraiva, and A. O'Garra, The regulation of IL-10 production by immune cells. Nat Rev Immunol 10 (2010) 170-81.
[93] K. Nagamatsu, A. Kuwae, T. Konaka, S. Nagai, S. Yoshida, M. Eguchi, M. Watanabe, H. Mimuro, S. Koyasu, and A. Abe, Bordetella evades the host immune system by inducing IL-10 through a type

III effector, BopN. J Exp Med 206 (2009) 3073-88.
[94] E.B. Wilson, and D.G. Brooks, The role of IL-10 in regulating immunity to persistent viral infections. Curr Top Microbiol Immunol 350 (2011) 39-65.
[95] S. Avdic, J.Z. Cao, B.P. McSharry, L.E. Clancy, R. Brown, M. Steain, D.J. Gottlieb, A. Abendroth, and B. Slobedman, Human cytomegalovirus interleukin-10 polarizes monocytes toward a deactivated M2c phenotype to repress host immune responses. J Virol 87 (2013) 10273-82.
[96] A. De Maria, M. Fogli, S. Mazza, M. Basso, A. Picciotto, P. Costa, S. Congia, M.C. Mingari, and L. Moretta, Increased natural cytotoxicity receptor expression and relevant IL-10 production in NK cells from chronically infected viremic HCV patients. Eur J Immunol 37 (2007) 445-55.
[97] J.K. Flynn, G.J. Dore, M. Hellard, B. Yeung, W.D. Rawlinson, P.A. White, J.M. Kaldor, A.R. Lloyd, R.A. Ffrench, and A.S. Group, Early IL-10 predominant responses are associated with progression to chronic hepatitis C virus infection in injecting drug users. J Viral Hepat 18 (2011) 549-61.
[98] E.A. Said, F.P. Dupuy, L. Trautmann, Y. Zhang, Y. Shi, M. El-Far, B.J. Hill, A. Noto, P. Ancuta, Y. Peretz, S.G. Fonseca, J. Van Grevenynghe, M.R. Boulassel, J. Bruneau, N.H. Shoukry, J.P. Routy, D.C. Douek, E.K. Haddad, and R.P. Sekaly, Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nat Med 16 (2010) 452-9.
[99] R. Lachmann, A. Loenenbach, T. Waterboer, N. Brenner, M. Pawlita, A. Michel, M. Thamm, C. Poethko-Muller, O. Wichmann, and M. Wiese-Posselt, Cytomegalovirus (CMV) seroprevalence in the adult population of Germany. PLoS One 13 (2018) e0200267.

[100] E. Adland, P. Klenerman, P. Goulder, and P.C. Matthews, Ongoing burden of disease and mortality from HIV/CMV coinfection in Africa in the antiretroviral therapy era. Front Microbiol 6 (2015) 1016.

[101] P.S. Redford, K.D. Mayer-Barber, F.W. McNab, E. Stavropoulos, A. Wack, A. Sher, and A. O'Garra, Influenza A virus impairs control of *Mycobacterium Tuberculosis* coinfection through a type I interferon receptor-dependent pathway. J Infect Dis 209 (2014) 270-4.

[102] K.D. Mayer-Barber, B.B. Andrade, S.D. Oland, E.P. Amaral, D.L. Barber, J. Gonzales, S.C. Derrick, R. Shi, N.P. Kumar, W. Wei, X. Yuan, G. Zhang, Y. Cai, S. Babu, M. Catalfamo, A.M. Salazar, L.E. Via, C.E. Barry, 3rd, and A. Sher, Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 511 (2014) 99-103.

[103] M.A. Freudenberg, T. Merlin, C. Kalis, Y. Chvatchko, H. Stubig, and C. Galanos, Cutting edge: a murine, IL-12-independent pathway of IFN-gamma induction by gram-negative bacteria based on STAT4 activation by Type I IFN and IL-18 signaling. J Immunol 169 (2002) 1665-8.

[104] S.A. Joosten, H.A. Fletcher, and T.H. Ottenhoff, A helicopter perspective on TB biomarkers: pathway and process based analysis of gene expression data provides new insight into TB pathogenesis. PLoS One 8 (2013) e73230.

[105] L.H. Kuller, R. Tracy, W. Belloso, S. De Wit, F. Drummond, H.C. Lane, B. Ledergerber, J. Lundgren, J. Neuhaus, D. Nixon, N.I. Paton, J.D. Neaton, and I.S.S. Group, Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS Med 5 (2008) e203.

[106] M. Hummel, and M.M. Abecassis, A model for reactivation of CMV from latency. J Clin Virol 25 Suppl 2 (2002) S123-36.
[107] L. Wittkop, J. Bitard, E. Lazaro, D. Neau, F. Bonnet, P. Mercie, M. Dupon, M. Hessamfar, M. Ventura, D. Malvy, F. Dabis, J.L. Pellegrin, J.F. Moreau, R. Thiebaut, I. Pellegrin, and S.e.A. Groupe d'Epidemiologie Clinique du, Effect of cytomegalovirus-induced immune response, self antigen-induced immune response, and microbial translocation on chronic immune activation in successfully treated HIV type 1-infected patients: the ANRS CO3 Aquitaine Cohort. J Infect Dis 207 (2013) 622-7.
[108] S. Gianella, C.M. Anderson, S.R. Var, M.F. Oliveira, S.M. Lada, M.V. Vargas, M. Massanella, S.J. Little, D.D. Richman, M.C. Strain, J. Perez-Santiago, and D.M. Smith, Replication of Human Herpesviruses Is Associated with Higher HIV DNA Levels during Antiretroviral Therapy Started at Early Phases of HIV Infection. J Virol 90 (2016) 3944-52.
[109] G.W. Wilkinson, A.J. Davison, P. Tomasec, C.A. Fielding, R. Aicheler, I. Murrell, S. Seirafian, E.C. Wang, M. Weekes, P.J. Lehner, G.S. Wilkie, and R.J. Stanton, Human cytomegalovirus: taking the strain. Med Microbiol Immunol 204 (2015) 273-84.
[110] M. Hosogai, N. Shima, Y. Nakatani, T. Inoue, T. Iso, H. Yokoo, H. Yorifuji, H. Akiyama, S. Kishi, and H. Isomura, Analysis of human cytomegalovirus replication in primary cultured human corneal endothelial cells. Br J Ophthalmol 99 (2015) 1583-90.
[111] S. Redpath, P. Ghazal, and N.R. Gascoigne, Hijacking and exploitation of IL-10 by intracellular pathogens. Trends Microbiol 9 (2001) 86-92.

Abstract in Korean

사람세포거대바이러스 (HCMV)는 β-herpesvirus에 속하여 전세계 인구의 약 60~80%가 감염되어 있다. 초기 감염의 경우에는 증상이 미미하지만 평생 잠복감염의 형태로 체내에 존재하기도 한다. 사람세포거대바이러스는 세포주기를 조절하고 사이토카인의 조절을 통하여 숙주의 면역 체계에 영향을 주게 되어 효율적인 감염의 형태를 조성하거나, 숙주 내 바이러스 확산이 가능한 환경을 구축한다. US2, 3, 11, UL111A 등과 같은 면역회피 단백을 암호화하고 있어 숙주의 신호전달과 활성을 조절하기도 한다. 이러한 숙주 면역 억제 환경에서는 숙주의 면역 체계가 병원균을 방어할 수 없는 상황이 될 수 있다. 사람세포거대바이러스가 감염된 숙주의 면역체계를 지배하게 되면 다른 병원체가 동일한 숙주에 감염될 수 있다. 이러한 예로, 사람세포거대바이러스와 면역결핍바이러스의 동시감염이나, 결핵의 동시감염 사례가 보고된 바 있다. 이러한 이유로, 사람세포거대바이러스가 잠복 감염된 상황에서 *Mycobacteroides abscessus* subspecies *massiliense* (*M. abscessus* subsp. *massiliense*)가 공동감염이 될 가능성이 더 높은지의 여부를 확인하고자 하였다.

비결핵항산균 (non-tuberculous mycobacteria, NTM) 중 강력한 약물내성을 갖는 종인 *Mycobateroides abscessus* (MAB) complex는 빠르게 증식하는 마이코박테리아로, 큰포식세포 내부에서 주로 증식한다. 사람세포거대바이러스의 감염이 *M. abscessus* subsp. *massiliense* 질병의 위험을 증가시키는지, 또는 사람세포거대바이러스 혈증의 소인이 *M. abscessus* subsp. *massiliense* 질병 발생 인자와 유사할 수 있는지의 여부는 알려진 바가 없다. 전사체 분석을 통해 큰포식세포가 두 병원체에 동시에 감염되었을 때 숙주의 IFN-r, TNF-a, IL-1의 면역 반응이 약해지고 *M. abscessus* subsp. *massiliense*에 대한 항박테리아성 면역반응이 줄어드는 것을 확인했다. 큰포식세포에 사람세포거대바이러스가 감염되었을 때 cmvIL-10의 분비가 증가하였고 이는 숙주세포 IL-10

의 신호 전달과 유사한 방식으로 숙주의 IL-10 수용체에 결합한다. 큰포식세포에 HCMV가 감염되었을 때 숙주의 IL-10 분비를 촉진하였고, IL-10 의존적 방식으로 *M. abscessus* subsp. *massiliense*의 증식을 촉진하였다. 바이러스 감염 후에 생산되는 cmvIL-10을 제거하면 숙주의 IL-10 생산이 감소하였고, 숙주의 IL-10을 중화시킨 경우에는 박테리아의 증식이 감소하는 결과를 확인하였다.

이러한 연구를 통해 HCMV 감염이 IL-10 분비를 증가시키고 *M. abscessus* subsp. *massiliense* 침입 시 숙주의 저하된 면역상태로 인해 동시 감염이 호발하는 현상을 설명할 수 있었다.

주요어: 사람세포거대바이러스, 인터루킨-10, 비결핵항산균, 큰포식세포, *Mycobacteroides abscessus* subspecies *massiliense*

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