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Mycoplasma pneumoniae–Specific IFN-γ–Producing CD4$^+$ Effector-Memory T Cells Correlate with Pulmonary Disease

To the Editor:

Mycoplasma pneumoniae (*Mp*) is a major cause of community-acquired pneumonia (CAP) in children (1). However, the pathogenesis of *Mp* CAP is not well understood. Lymphocyte responses against *Mp* have been reported to promote either protection or immunopathology in mice (1, 2). In humans, intradermal injection of *Mp* antigen elicited a delayed-type hypersensitivity skin reaction in patients with *Mp* infection (3). The size of the delayed-type hypersensitivity skin induration, which depends mainly on infiltrating CD4$^+$ T helper 1 (Th1) cells, correlated with the severity of pulmonary infiltrates in those patients (3). These observations suggest that the *Mp*-specific T-cell response contributes to *Mp* pulmonary disease.

We showed that the measurement of specific IgM antibody-secreting cells (ASCs) in blood discriminated patients with CAP with *Mp* infection from *Mp* carriers suffering from CAP caused by other pathogens (4). Using this well-diagnosed cohort, we here investigated the *Mp*-specific T-cell response and its contribution to pulmonary disease.

Children with CAP ($n = 35$) and healthy controls (HCs; $n = 16$) aged 3–18 years from a prospective longitudinal study (4, 5) from which peripheral blood mononuclear cells (PBMCs) were available were included in this study. Baseline characteristics of subjects are shown in Table E1 in the data supplement. The study was approved by the ethics committee of Zurich, Switzerland (no. 2016-00148). Detailed methods are shown in the data supplement. CAP disease severity was assessed based on chest radiograph (CXR) findings, hypoxemia (oxygen saturation as measured by pulse oximetry <93%) requiring oxygen supply, and inflammatory parameters (6). CXRs were graded with an adapted CXR severity scoring system (7), with grades 1, 2, and 3 representing increasing severity (Table E2 and Figure E1).

We first developed an *Mp*-specific IFN-γ enzyme-linked immunospot (ELISpot) assay (data supplement (5, 8)) and demonstrated its specificity by comparing patients with *Mp* PCR-positive (*Mp*$^+$) CAP and *Mp*$^+$ HCs (carriers), as well as patients with *Mp* PCR-negative (*Mp*$^-$) CAP and *Mp*$^-$ HCs (Figure 1A). The ELISpot assay detected IFN-γ released by PBMCs after stimulation with *Mp* antigen most frequently and pronounced in patients with *Mp*$^+$ CAP (Figures 1A and 1B). This is in line with IgM ASC ELISpot assay results, which confirmed *Mp* infection in those patients with *Mp*$^+$ CAP (Table E1). However, in contrast to IgM ASCs, which were short lived (5) and mainly present during the symptomatic stage (≤20 days after onset of symptoms), the *Mp*-specific IFN-γ response was significantly longer lasting and also detectable in the convalescent stage (>20 days) ($P = 0.0007$) (Figure 1C).

To identify *the* IFN-γ–producing cells, we depleted CD4$^+$ or CD8$^+$ T cells from PBMCs of a patient with *Mp*$^+$ CAP (Figure E2). Depletion of CD4$^+$ T cells reduced IFN-γ spot-forming units (SFUs) by 96% and 88% upon 24 hours and 48 hours preincubation with *Mp* antigen, respectively (Figure 1D). CD8 depletion did not markedly reduce IFN-γ SFUs. These findings were corroborated by flow cytometry: only IFN-γ–producing CD4$^+$ T cells, and almost no CD8$^+$ T cells, were detected (Figure 1E). Among these IFN-γ–producing CD4$^+$ T cells, a significant proportion coexpressed CD69 and CD40L, identifying antigen-responsive T cells (data not shown). Importantly, the majority of IFN-γ$^+$CD4$^+$ T cells were detected in the effector-memory T cell (T_{EM}) compartment (Figures 1F and E3).

Th1 cells have been reported to contribute to immune-mediated tissue damage in other infectious diseases (9–14). Therefore, we correlated the *Mp*-specific IFN-γ response with disease severity in *Mp*$^+$ CAP (Table E3). The extent of pulmonary disease reflected by increased CXR grading correlated positively with the degree of the specific IFN-γ response in symptomatic ($R = 0.49$, $P = 0.03$) and convalescent stage ($R = 0.62$, $P = 0.006$) (Figures 1G and 1H). Interestingly, in contrast to patients with CXR grade 1, those with CXR grades 2 and 3 showed even an increase in IFN-γ–producing cells over time (Figure 1I). The IFN-γ response was antigen dose dependent and most pronounced for patients with CXR grade 3 (Figures 1J and E4A). However, the IFN-γ response did not correlate with bacterial load in the upper respiratory tract. No relation was observed between CXR grading and bacterial load (Figure E4B) or the *Mp*-specific B-cell response (Figure E5). The acute IFN-γ response was also associated with C-reactive protein levels ($P = 0.009$; Figure 1K) and oxygen need

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This letter has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.

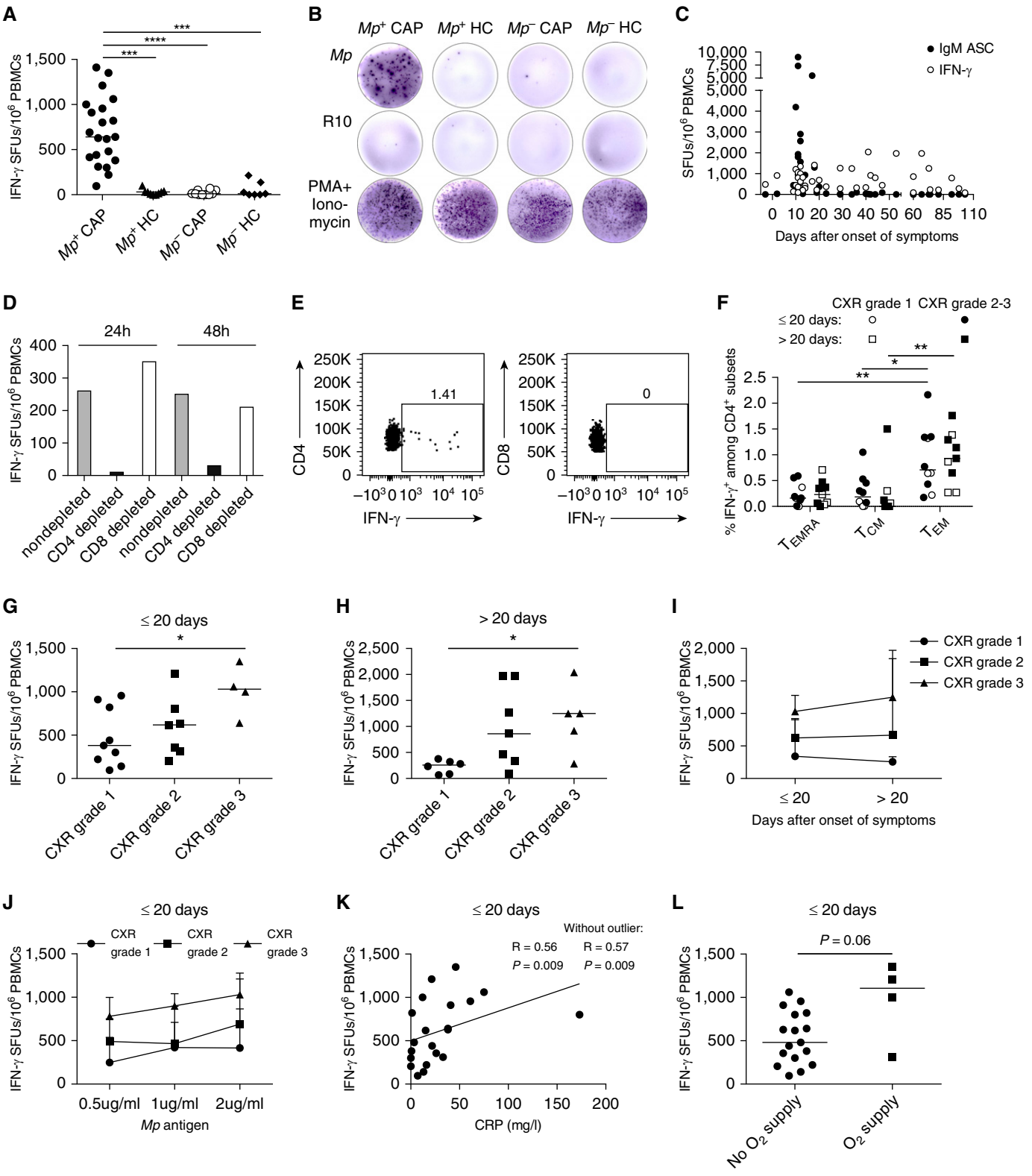

Figure 1. *Mp*-specific IFN-γ response by CD4$^+$ T cells. (*A* and *B*) *Mp*-specific IFN-γ SFUs per 10^6 PBMCs (*A*) and representative patterns (*B*) in ELISpot assay of *Mp*$^+$ CAP ($n = 21$ of the total 25 patient samples in Table E1 [$n = 1$ exclusively used for flow cytometry in *E* and *F*; $n = 3$ only available at the convalescent stage]; samples collected at median 12 days [interquartile range, 11–16] after symptom onset), *Mp*$^+$ HC (carrier, $n = 9$), *Mp*$^-$ CAP ($n = 10$),

($P = 0.06$; Figure 1L). In contrast to the IFN-γ response (Figure 1I), C-reactive protein returned to normal levels in all patients at the convalescent stage (Figure E4C).

To our knowledge, these are the first data indicating that $CD4^+$ T_{EM} cells form the major population of the pathogen-specific IFN-γ response in children with *Mp* CAP, and that the presence of these Th1 cells in peripheral blood correlates with pulmonary disease severity.

The IFN-γ ELISpot assay is one of the most sensitive *ex vivo* detection methods for pathogen-specific T cells (8). Here, we demonstrate high specificity of the *Mp*-specific IFN-γ ELISpot assay in a well-diagnosed cohort of patients with CAP and healthy controls (4, 5). Interestingly, the detection of the IFN-γ response by ELISpot assay allowed also a differentiation between *Mp* infection and carriage. However, in contrast to the IgM ASC response, which is short-lived and associated with clinical disease (5), the long-lasting nature of the *Mp*-specific IFN-γ response may pose a limitation to the IFN-γ ELISpot assay as diagnostic test for *Mp* infection.

Our findings on the *Mp*-specific Th1 cell response are corroborated by previous observations in animal models suggesting that Th1 cells contribute to inflammatory lesions in mycoplasma pneumonia (1, 2, 15), and clinical studies in children and adults where the IFN-γ response correlated with the disease severity and/or radiological changes in CAP associated with *Mp* (16–18). Furthermore, we expand these observations by revealing *Mp*-specific T_{EM} cells as the major Th1 cell compartment associated with more severe disease. In fact, Th1-mediated immunopathology has been proposed to play a role in other infectious diseases (9–14). T_{EM} cells migrate to inflamed peripheral tissues and display immediate effector function (19, 20). The higher INF-γ response in patients with *Mp* CAP with severe disease, which even increased over time despite bacterial clearance, points to dysregulation and expansion of effector-memory Th1 cells rather than to a more pronounced or persistent triggering by *Mp* antigens.

In conclusion, these data further support the hypothesis that host cell-mediated immunity, particularly pathogen-specific IFN-γ–producing $CD4^+$ T_{EM} cells, is involved in the pathogenesis of *Mp* CAP. Further studies are required to reveal the exact role of these cells in *Mp* pulmonary disease. ■

Author disclosures are available with the text of this letter at www.atsjournals.org.

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Elena Pánisová, Ph.D.*
University Children's Hospital Zurich
Zurich, Switzerland

Wendy W. J. Unger, Ph.D.*
Erasmus MC University Medical Center–Sophia Children's Hospital
Rotterdam, the Netherlands

Christoph Berger, M.D.
Patrick M. Meyer Sauteur, M.D., Ph.D.‡
University Children's Hospital Zurich
Zurich, Switzerland

ORCID IDs: 0000-0002-8489-8406 (E.P.); 0000-0001-9484-261X (W.W.J.U.); 0000-0002-2373-8804 (C.B.); 0000-0002-4312-9803 (P.M.M.S.).

*These authors contributed equally to this work.
‡Corresponding author (e-mail: patrick.meyer@kispi.uzh.ch).

Figure 1. (*Continued*). and Mp^- HC ($n = 7$) (100,000 PBMCs per well). (*C*) *Mp*-specific IgM ASC (filled symbols) or IFN-γ (empty symbols) SFUs per 10^6 PBMCs by ELISpot assay in relation to days after onset of symptoms ($n = 41$ Mp^+ CAP patient samples; $n = 21$ during symptomatic stage [≤20 days after onset of symptoms] and $n = 20$ in convalescent stage [>20 days after onset of symptoms]). (*D*) IFN-γ SFUs per 10^6 PBMCs (ELISpot assay) of a patient with Mp^+ CAP without depletion (gray bars) or with depletion of $CD4^+$ (black bars) and $CD8^+$ (white bars) T cells. PBMCs were preincubated for 24 hours and 48 hours with *Mp* antigen. (*E*) Representative flow cytometry dot plots of *Mp*-specific $CD4^+IFN\text{-}\gamma^+$ and $CD8^+IFN\text{-}\gamma^+$ T_{EM} cells of a patient with Mp^+ CAP at the symptomatic stage. The percentages of $IFN\text{-}\gamma^+$ cells are indicated. (*F*) IFN-γ–producing memory $CD4^+$ T-cell subsets measured by flow cytometry of patients with Mp^+ CAP during symptomatic stage (≤20 d, $n = 10$; circles) and convalescent stage (>20 d, $n = 9$; squares), and in relation to CXR grade 1 (white symbols) and grade 2–3 (black symbols). $CD4^+$ T-cell subsets were stained with antibodies binding to CD45RA and CCR7 (T_{EMRA}: $CD45RA^+CCR7^-$; T_{CM}: $CD45RA^-CCR7^+$; T_{EM}: $CD45RA^-CCR7^-$). There were no statistically significant differences between percentage of $IFN\text{-}\gamma^+$ cells and CXR grading per subset and stage of disease. (*G–I*) *Mp*-specific IFN-γ SFUs per 10^6 PBMCs of patients with Mp^+ CAP in relation to CXR grading (grades 1, 2, and 3 represent increasing severity) during symptomatic stage (≤20 d) ($n = 20$ of the 21 patients in *A–C* with acute sample and also CXR available) (*G*), convalescent stage (>20 d) ($n = 18$ of the 20 patients in *C* with convalescent sample and also CXR available) (*H*), and over time ($n = 16$ patients with both acute and convalescent sample and also CXR available) (*I*). (*J*) Antigen dose effect on IFN-γ response upon prestimulation for 24 hours with 0.5 μg/ml, 1 μg/ml, and 2 μg/ml antigen during symptomatic stage (≤20 d) from patients with CXR grade 1 ($n = 7$), grade 2 ($n = 6$), and grade 3 ($n = 4$). (*K* and *L*) *Mp*-specific IFN-γ SFUs per 10^6 PBMCs of patients with Mp^+ CAP ($n = 21$), preincubated with 2 μg/ml of *Mp* antigen for 24 hours, and assessed on the basis of CRP levels (*K*) or need for oxygen supply (*L*) during symptomatic stage (≤20 d). Horizontal lines (*A*, *F–H*, and *L*) or symbols (*I* and *J*) indicate median values and whiskers extend to the first and third quartile. Statistical significance was determined by Kruskal-Wallis test with *post hoc* Dunn's multiple comparisons test (*A* and *F–J*), Mann-Whitney *U* test (*L*), or Spearman rank correlation (*K*). The CXR grades (1–2–3) were used as numerical values for statistical analysis. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$; only statistically significant differences are indicated in the graphs.
ASC = antibody-secreting cell; CAP = community-acquired pneumonia; CRP = C-reactive protein; CXR = chest radiograph; HC = healthy control; *Mp* = *Mycoplasma pneumoniae*; PBMC = peripheral blood mononuclear cell; R10 = complete RPMI; SFU = spot-forming unit; T_{CM} = central-memory T cell; T_{EM} = effector-memory T cell; T_{EMRA} = terminally differentiated effector-memory T cell.

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Lung Gene Expression Analysis Web Portal Version 3: Lung-at-a-Glance

To the Editor:

Recent advances in single-cell omics have provided increasing insights into the pathogenesis of human diseases, including those affecting the lung (1–7). The density of omics data relevant to lung biology and diseases is increasing exponentially through the work of research consortia and individual investigators (1, 3, 8–12). Discerning the best way to optimize the use of these rich datasets, integrate multiomics data, extract biologically meaningful knowledge, and make that knowledge available to the research community in a user-friendly manner is a challenging opportunity. With support from the National Heart, Lung, and Blood Institute (NHLBI) "LungMAP" (Lung Map) consortium, we developed the Lung Gene Expression Analysis (LGEA) database and web portal to facilitate access and visualization of extensive bulk, sorted, single-cell transcriptomic and image data from human and mouse lungs at different stages of development and disease (13, 14). Data hosted on LGEA are primarily produced by LungMAP research centers. We process and interpret the data and make it available to all investigators before its publication (8). LGEA has been widely used by researchers from more than 130 institutions from 52 different countries and has been cited in more than 130 scientific publications. The newly updated LGEA version 3 introduces a new featured web toolset, "lung-at-a-glance," for exploring and understanding complex multiomics and imaging data, providing an interactive web interface to bridge lung anatomic ontology classifications to lung structure, histology, and immunofluorescence confocal images and cell type–specific gene expression.

Lung-at-a-glance consists of "region," "cell," and "gene," three interactive components all designed to provide data access with a single click on the icons (https://research.cchmc.org/pbge/lunggens/tools/lung_at_glance.html). We name the toolset as

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Author Contributions: Y.D., M.G., and Y.X. conceived and designed the web application. Y.D. developed the database and web application of Lung Gene Expression Analysis web portal. W.O. developed the web application of Lung Gene Expression Analysis lung ontology. Y.D. and W.O. developed the lung-at-a-glance toolsets. J.A.K. and J.A.W. designed and developed the web application of lung image. Y.D., M.G., S.Z., and Y.X. contributed to data analysis and interpretation. Y.D., J.A.W., and Y.X. wrote the manuscript. All authors contributed to the manuscript editing and approved the final manuscript.

This letter has a data supplement, which is accessible from this issue's table of contents at www.atsjournals.org.