Do Mucosa-associated Invariant T Cells Checkmate *Streptococcus pneumoniae*?

Streptococcus pneumoniae is not only a common pathogen for community-acquired pneumonia, it can also cause a wide variety of illnesses such as otitis media and invasive pneumococcal disease, including meningitis and bacteremia (1). Invasive pneumococcal disease can be extremely serious, and pneumococcal conjugate vaccines were developed to reduce the burden of disease caused by multiple pneumococcal serotypes (2). However, nonvaccine serotypes, such as 19A, remain considerable threats for invasive pneumococcal disease (3).

In addition to the important role of the adaptive immune system in eliminating invading bacterial pathogens, cells of the innate immune system offer an immediate, decisive line of defense (4, 5). Among these cells, mucosa-associated invariant T (MAIT) cells specifically recognize metabolites of the riboflavin biosynthesis pathway presented in the context of a major histocompatibility complex class I–related molecule (MR1) (6–8). MAIT cells have been identified in both humans and rodents, and a role for MAIT cells in the defense against a range of bacterial pathogens that infect the respiratory tract, such as *Mycobacterium tuberculosis*, *Klebsiella pneumoniae*, and *Francisella tularensis*, as well as fungal pathogens, has been described (9, 10). In this issue of the *Journal*, Hartmann and colleagues (pp. 767–776) show for the first time the ability of MAIT cells to recognize and kill *S. pneumoniae*–infected airway epithelial cells (AECs) (11). However, the response of the MAIT cells to different clinical isolates of serotype 19A was not uniform.

In humans, MAIT cells comprise 1–10% of T cells in the peripheral blood, 3–7% of T cells in the lamina propria in the gut, and 10–20% of T cells in the lung (8). MAIT cells express an αβ T-cell antigen receptor comprising a near-invariant α chain coupled with a restricted set of β chains that are relatively conserved in humans and mice (12). Additional surface markers characteristic of resting MAIT cells in healthy adults include high levels of the C-type lectin CD161 and the IL-18 receptor α subunit. These cells express transcription factors such as promyelocytic leukemia zinc finger, RAR-related orphan receptor γ, T-bet, and eomesodermin, which are associated with effector T cells (13). Upon engagement by their ligands (riboflavin metabolites), MAIT cells rapidly release cytokines such as IFN-γ, TNF, and IL-17, and also use granzymes to exert cytolytic activity against infected cells (8, 14).

Hartmann and colleagues examined 35 clinical isolates of *S. pneumoniae* serotype 19A isolated from different compartments of the body for their ability to activate a MAIT cell clone, D426 G11. Although human monocyte-derived dendritic cells (DCs) were infected by the different clinical isolates at the same multiplicity of infection, IFN-γ production from the MAIT cell clone upon coculture with the infected DCs was found to vary among the isolates. One isolate, SP9, consistently triggered a high IFN-γ response, whereas another, SP37, induced a much lower cytokine response, with only 18 of the 35 isolates being able to promote IFN-γ production above background levels. IFN-γ production was completely dependent on MR1, but no correlation was found between IFN-γ produced by MAIT cells and cytokines produced by the infected DCs, such as IL-12 and TNF-α. No IL-18 was detected from the infected DCs. There was also no difference in the ability of the DCs to phagocytose and kill isolate SP9 versus SP37.

MAIT cells recognize different metabolites in the riboflavin biosynthesis pathway. The riboflavin precursor 5-amino-6-ribityl-uracil (5-A-RU) can combine with glyoxal or methylglyoxal from the host or bacterial cells to form the unstable metabolites 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribityluracil (5-OP-RU), which are stabilized by MR1 and serve as potent MAIT cell–specific antigens (7). In the study by Hartmann and colleagues, the level of expression of RibD, one of the enzymes involved in riboflavin metabolism, was found to correlate with the IFN-γ response in MAIT cells (11). Although the authors failed to detect the specific ligands 5-OE-RU and 5-OP-RU by mass spectrometry, the relative levels of riboflavin and the downstream molecule flavin mononucleotide were significantly higher in the isolate SP9 than in SP37. Furthermore, changing the availability of riboflavin in the culture medium altered the capacity of SP9 and SP37 to activate the MAIT cell clone, with increasing levels of exogenously supplied riboflavin decreasing MAIT cell activation. An important revelation in this study is that *S. pneumoniae*–infected AECs can be targeted by MAIT cells (11). Whereas SP9-infected AECs were able to promote IFN-γ production from the MAIT cell clone, limiting bacterial survival, SP37 failed to show any of these effects (11).

The authors also performed experiments using Vα19 transgenic mice on a TCR-α–deficient background to facilitate the accumulation of more tetramer-positive cells in the lungs. The same isolates, SP9 and SP37, were allowed to infect the transgenic mice, although serotype 19A was previously described to be avirulent in mice. As observed in human cells, a differential cytokine response was elicited in mice, with SP9 inducing significantly more IFN-γ and IL-17 in tetramer-positive cells than SP37, although infection was similarly controlled in both strains. Although these results showed that different clinical isolates of serotype 19A have a differential ability to induce cytokine production by MAIT cells *in vivo* as well, whether MAIT cell cytokine response has any impact on invasive disease caused by pneumococci remains to be determined. Because a virulent strain of the bacterium also induced cytokine production from MAIT cells, it would be interesting to determine whether this strain induces a worse outcome in the absence of MAIT cell function in $MR1^{-/-}$ mice.

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In a different study published recently, variable cytokine response to two different pathogens, *Escherichia coli* and *Candida albicans*, was demonstrated (15). The cytokine response to *E. coli* was more robust with greater TCR downregulation than that to *C. albicans*. The IFN-γ response in mice after infection with both *E. coli* and *C. albicans* was mostly, but not exclusively, dependent on MAIT cells, whereas TNF-α production was totally MAIT cell dependent. Also, a modest TCR-β bias was noted in the differential sensitivity to the bacterial versus fungal pathogens. Collectively, and in the context of the literature, the findings in the present study suggest that despite sequence conservation in MAIT cells, the functional difference in response to different pathogens depends on the level and type of ligand generated in the infected cell. However, it is challenging to assay free MR1 ligands that are intermediates of the riboflavin pathway because of their unstable, transient nature. In summary, MAIT cells display a functional heterogeneity in response to clinical isolates of *S. pneumoniae* that cause invasive disease, and an inadequate MAIT cell response may be a determinant in the development of invasive disease. ■

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References

1. Tuomanen EI, Austrian R, Masure HR. Pathogenesis of pneumococcal infection. *N Engl J Med* 1995;332:1280–1284.
2. Singleton RJ, Hennessy TW, Bulkow LR, Hammitt LL, Zulz T, Hurlburt DA, *et al*. Invasive pneumococcal disease caused by nonvaccine serotypes among Alaska native children with high levels of 7-valent pneumococcal conjugate vaccine coverage. *JAMA* 2007;297: 1784–1792.
3. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA* 2007;298:1772–1778.
4. Iwasaki A, Medzhitov R. Control of adaptive immunity by the innate immune system. *Nat Immunol* 2015;16:343–353.
5. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. The burgeoning family of unconventional T cells. *Nat Immunol* 2015;16: 1114–1123.
6. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, *et al*. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. *Nature* 2003;422:164–169.
7. Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, *et al*. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. *Nature* 2014;509:361–365.
8. Salou M, Franciszkiewicz K, Lantz O. MAIT cells in infectious diseases. *Curr Opin Immunol* 2017;48:7–14.
9. Georgel P, Radosavljevic M, Macquin C, Bahram S. The non-conventional MHC class I MR1 molecule controls infection by *Klebsiella pneumoniae* in mice. *Mol Immunol* 2011;48:769–775.
10. Meierovics A, Yankelevich WJ, Cowley SC. MAIT cells are critical for optimal mucosal immune responses during in vivo pulmonary bacterial infection. *Proc Natl Acad Sci USA* 2013;110:E3119–E3128.
11. Hartmann N, McMurtrey C, Sorensen ML, Huber ME, Kurapova R, Coleman FT, *et al*. Riboflavin metabolism variation among clinical isolates of *Streptococcus pneumoniae* results in differential activation of mucosal-associated invariant T cells. *Am J Respir Cell Mol Biol* 2018;58:767–776.
12. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, *et al*. Stepwise development of MAIT cells in mouse and human. *PLoS Biol* 2009;7:e54.
13. Savage AK, Constantinides MG, Han J, Picard D, Martin E, Li B, *et al*. The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* 2008;29:391–403.
14. Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, *et al*. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. *Mucosal Immunol* 2015;8: 429–440.
15. Dias J, Leeansyah E, Sandberg JK. Multiple layers of heterogeneity and subset diversity in human MAIT cell responses to distinct microorganisms and to innate cytokines. *Proc Natl Acad Sci USA* 2017;114:E5434–E5443.