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Review

Mycobacterium tuberculosis-specific and MHC class I-restricted CD8+ T-cells exhibit a stem cell precursor-like phenotype in patients with active pulmonary tuberculosis

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SUMMARY

The nature and longevity of the T-cell response directed against *Mycobacterium tuberculosis* (MTB) are important for effective pathogen containment. We analyzed ex vivo the nature of MTB antigen-specific T-cell responses directed against the MTB secreted antigens Rv0288, Rv1886c, Rv3875, the antigens Rv2958c, Rv2957, and Rv0447c (intracellular, non-secreted enzymes) in blood from Korean patients with active tuberculosis (TB). MTB-specific T-cell function was defined by intracellular cytokine production (interleukin (IL)-2, interferon gamma, tumour necrosis factor alpha, and IL-17) and by multimer-guided (HLA-A*02:01 and HLA-A*24:02) analysis of epitope-specific CD8+ T-cells, along with phenotypic markers (CD45RA and CCR7), CD107a, a marker for degranulation, and CD127 co-staining for T-cell differentiation and homing. Cytokine production analysis underestimated the frequencies of MTB antigen-specific T-cells defined by major histocompatibility complex (MHC) class I–peptide multimer analysis. We showed that MTB antigen-specific CD8+ T-cells exhibit a distinct marker profile associated with the nature of the MTB antigens, i.e., Rv0288, Rv1886c, and Rv3875-reactive T-cells clustered in the precursor T-cell compartment, whereas Rv2958c, Rv2957, and Rv0447c-reactive T-cells were associated with the terminally differentiated T-cell phenotype, in the patient cohort. Rv0288, Rv1886c, and Rv3875-specific CD8+ T-cells were significantly enriched for CD107a+ T-cells in HLA-A*02:01 ($p < 0.0001$) and HLA-A*24:02 ($p = 0.0018$) positive individuals, as compared to Rv2958c, Rv2957, and Rv0447c antigens. CD127 (IL-7 receptor)-expressing T-cells were enriched in HLA-A*02:01-positive individuals for the Rv0288, Rv1886c, and Rv3875 specificities ($p = 0.03$). A high proportion of antigen-specific T-cells showed a precursor-like phenotype (CD45RA+CCR7+) and expressed the stem cell-associated markers CD95 and c-kit. These data show that MTB-specific T-cells can express stem cell-like features; this is associated with the nature of the MTB antigen and the genetic background of the individual.

1. Introduction

Mycobacterium tuberculosis (MTB), the aetiological agent of tuberculosis (TB) affects 8.8 million people and causes an estimated 1.5 million deaths globally per annum.[1] Several factors such as poor BCG vaccine efficacy, challenges in making an accurate diagnosis of TB using available diagnostics, widespread emergence of drug resistance, poor infection control measures, and co-infection with HIV, continue to fuel the TB epidemic.[2–5] Critical to determining protective immune responses is the study of multiple MTB exposures, recurrent MTB re-infections, as well as simultaneous infections with multiple MTB strains.[6] The immunological consequences of these scenarios and their impact on memory immune responses have not yet been examined. A better understanding of protective or ineffective immune responses in TB may aid the design of biologically relevant biomarkers to visualize clinically relevant memory immune responses.

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Different T-cell subsets, including CD4+ and CD8+ T-cells, play a crucial role in MTB containment,[7,8] i.e., by cytokine production or direct cytotoxicity. Th1 cytokines, interferon gamma (IFN-γ) and tumour necrosis factor alpha (TNF-α), are important for optimal MTB control.[8,9] Polyfunctionality of T-cells, i.e., the ability to simultaneously produce interleukin 2 (IL-2), IFN-γ, and TNF-α, has previously been linked to immune protection and/or post-vaccination memory responses in different infectious diseases including HIV.[10] However, in TB, the data available to date are pointing in different directions.[11–14]

T-cells recognize, and are activated by, small parts (peptide stretches) of protein-derived antigens presented via major histocompatibility complex (MHC) glycoproteins (in humans called human leukocyte antigen (HLA)) on antigen-presenting cells (APCs). Many different immunogenic MTB proteins have been identified by their ability to induce antigen-specific T-cell responses, usually defined by cytokine production. Such MTB proteins include the early expressed (secreted) antigens Ag85B (Rv1886c), TB10.4 (Rv0288), and ESAT-6 (Rv3875).[15–19] More recently, we have identified epitopes and T-cell responses (in both CD4+ and CD8+ T-cells) provided from (non-secreted) MTB intracellular enzymes (cyclopropane fatty acid synthase (CFA synthase; Rv0447c) and two glycosyltransferases (Rv2957 and Rv2958)[19–21]), which are preferentially expressed in slow-growing bacteria, including in *Mycobacterium* species. Rv0447c is a key enzyme involved in MTB lipid metabolism, producing factors that make the cell wall impermeable.[22] The two glycosyltransferases belong to the intermediary metabolism; they are involved in the formation of phenolic glycolipids and glycosylated *p*-hydroxybenzoic acid methyl esters, constituents of the mycobacterial cell envelope and MTB virulence factors.[23] Rv2958c is also believed to be involved in the ability of MTB to survive inside macrophages.[24] These (non-secreted) proteins may show a different pattern of immunogenicity compared to the early expressed (secreted) MTB antigens, yet this has not been formally shown.

Antigen-specific T-cells recognizing MTB antigens have been identified by different immune readouts,[1] for example by using cytokine production, i.e., IFN-γ or TNF-α (by intracellular cytokine staining (ICS), ELISPOT, or ELISA in cell culture supernatant after in vitro stimulation with defined molecular targets). Alternatively, epitope-specific T-cells can directly be identified ex vivo, without the need for functional readouts, using soluble MHC class I–peptide complexes (i.e., MHC multimers). One advantage of this approach is the possibility to assess T-cell frequencies without any in vitro manipulation,[25] in combination with cell-surface markers to determine the phenotype and effector functions of the pathogen-specific T-cells. Based on the cell-surface markers CD45RA and CCR7, mature T-cells can be divided into four different phenotypic compartments. Precursor T-cells express both CD45RA and CCR7; they replenish the T-cell pool, yet produce only limited amounts of cytokines. Central memory T-cells (CD45RA−CCR7+) represent lymph node homing antigen-experienced cells that lack immediate effector functions (except IL-2 production). The third and fourth compartments represent effector memory T-cells (which have down-regulated both cell-surface markers) and terminally differentiated effector cells (CD45RA+CCR7−), which home to anatomical sites of disease.[26,27] The latter populations represent T-cell populations producing IFN-γ and TNF-α with cytotoxic potential.[28]

In the current study, we compared the frequency of MTB antigen-specific CD8+ T-cells in peripheral blood mononuclear cells (PBMCs) detected by ICS to the frequency of antigen-specific T-cells directed to defined MTB epitopes using MHC class I multimers, in order to evaluate whether antigen-specific T-cells are possibly being underestimated in active pulmonary TB. We characterized homing, differentiation, and effector functionality (CD107a, degranulation), IL-7 receptor (CD127)-mediating survival signals, as well as c-kit (CD117) and CD95, 'stem-ness', in T-cell populations in association with the MHC class I genetic background and the nature of the MTB antigens (i.e., intracellular (non-secreted) enzyme antigens vs. MTB secreted antigens). The results show in which T-cell compartment MTB-specific T-cells reside; a crucial information since the lack of effector T-cells (CD45RA+ CCR7−) has been associated with an increased risk of developing active TB.[8]

2. Materials and methods

2.1. Patient data

Twelve newly diagnosed patients with active pulmonary TB (acid-fast and culture-positive) at St. Mary's Hospital, Seoul, South Korea were enrolled in the study. Eleven patients were male and one was female (patient 12), and they ranged in age from 23 to 73 years (**Supplementary Material**, Table S1). The samples were obtained after diagnosis and after drug susceptibility testing (DST), which were performed in accordance with international guidelines. Institutional review board (IRB) consent was obtained from the Catholic Medical Centre, Seoul, South Korea (Ref. XC09FZZZ0046K; the independent ethics review committee of the Catholic University Seoul, South Korea) and from the ethics committee in Stockholm (Ref. 2011/863-31/2; Stockholm City Ethical Council South Committee). The ethics committees reviewed the study plan, as well as the patient informed consent forms, which are on file with the institutions as stipulated. The patients provided written consent, these papers are on file at the hospital.

Blood samples were drawn from the patients and PBMCs were isolated. The PBMCs were HLA-typed in South Korea for the alleles HLA-A*02 and A*24. Three individuals tested positive for HLA-A*02, three individuals positive for HLA-A*24, and six individuals double-positive for both alleles were included in order to gauge MHC class I multimer complexes. We chose to use material from patients with newly diagnosed active pulmonary TB, since the clinical definition of the spectrum of latent TB is challenging.

2.2. Selection of epitopes for multimer construction

Epitopes from different well-characterized MTB proteins (Rv3875, Rv0288, and Rv1886c) for multimer construction were selected based on (1) previously reported detection of antigen-specific T-cells (A2-Rv3875$_{AMASTEGNV}$,[29] A2-Rv3875 $_{LLDEGKQSL}$,[30] A2-Rv0288$_{IMYNYPAML}$,[31] A24-Rv0288$_{IMYNYPAML}$,[31] A2-Rv1886c$_{KLVANNTRL}$,[17] A2-Rv1886c$_{FIYAGSLSA}$,[17] and A24-Rv1886c$_{IYAGSLSAL}$[17]), and (2) MHC peptide binding data (A24-Rv3875$_{AYQGVQQKW}$ (our own non-published data) and A24-Rv3875$_{ELNNALQNL}$[32]). Epitopes from proteins Rv2957, Rv2958c, and Rv0447c were selected based on high SYFPEITHI scores,[33] which translate into a high likelihood of successful soluble MHC class I antigen–peptide production (A2-Rv2957$_{SIIIPTLNV}$ (score = 26), A24-Rv2957$_{PYNLRYRVL}$ (score = 21), A2-Rv2958c$_{ALADLPVTV}$ (score = 30), A24-Rv2958c$_{KYIAADRKI}$ (score = 25), A2-Rv0447c$_{VLAGSVDEL}$ (score = 31), and A24-Rv0447c$_{KYIFPGGLL}$ (score = 25)).

2.3. Cellular analysis with multimers

Fifteen different MHC class I–peptide multimers were either purchased or constructed in our laboratory: A2-Rv3875$_{AMASTEGNV}$, A2-Rv3875$_{LLDEGKQSL}$, A2-Rv1886c$_{KLVANNTRL}$, A24-Rv1886c$_{IYAGSLSAL}$ (Beckman Coulter, San Diego, CA, USA), A24-Rv3875$_{AYQGVQQKW}$, A24-Rv3875$_{ELNNALQNL}$, A2-Rv2957$_{SIIIPTLNV}$, A24-Rv2957$_{PYNLRYRVL}$, A2-Rv2958c$_{ALADLPVTV}$, A24-Rv2958c$_{KYIAADRKI}$, A2-Rv0447c$_{VLAGSVDEL}$, A24-Rv0447c$_{KYIFPGGLL}$ (Immudex, Copenhagen, Denmark), A2-Rv0288$_{IMYNYPAML}$, A24-Rv0288$_{IMYNYPAML}$, and A2-Rv1886c $_{FIYAGSLSA}$