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Review

Mycobacterium-Specific Human CD8 T Cell Responses

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Abstract. Tuberculosis (TB) remains a global health problem. There is an intense effort to identify correlates of protective immunity and to design new TB vaccines. CD8 T cells are thought to play a significant role in controlling *Mycobacterium tuberculosis* infection. Relatively little has been published about the antigens and epitopes targeted by mycobacteria-specific CD8 T cells. Here we present an update of our 1999 overview of human CD8 T cell epitopes in mycobacterial antigens and discuss related issues relevant to TB diagnosis and vaccine development.

Key words: mycobacterium; tuberculosis; CD8; epitope; vaccine; diagnosis.

Introduction

In 1993 the World Health Organization (WHO) declared tuberculosis (TB) a global health emergency. It is estimated that over the next two decades, nearly one billion people will become infected with *Mycobacterium tuberculosis*, 200 million people will get sick, and 35 million will die from TB if control is not further strengthened[53]. A significantly increased incidence of TB among HIV-1-infected individuals strongly suggests that cellular immunity is important for protection against TB, but the actual correlates of protection remain ill-defined.

There is mounting evidence from animal studies that CD8 T cells are involved in the control of latent *M. tuberculosis* infection[52]. Studies in gene knockout mice (e.g. CD8α, β_2-microglobulin, transporter-associated-with-antigen-processing 1 (TAP1)) have clearly shown that CD8 T cells can delay progression to disease (reviewed in refs.[15, 43]). However, relatively little has been published on the functional role of mycobacteria-specific CD8 T cells in humans or on the actual mycobacterial antigens and epitopes targeted by these killer cells. Here we present an update of our 1999 overview of human CD8 T cell epitopes in mycobacterial antigens and discuss related issues relevant to TB diagnosis and vaccine development[21].

Update of Human CD8 T Cell Epitopes in Mycobacterial Antigens

Current listing of epitopes

Human CD8 T cell epitopes have so far been found in 12 out of 3924 mycobacterial open reading frames (ORFs) of *M. tuberculosis*, with HLA-restriction elements limited to only 6 alleles: A*0201, A*6802, B*14, B*3501, B*44 and B*52 (Table 1). The majority of CD8 T cell epitopes are in secreted antigens, including Ag85 complex proteins, ESAT-6, CFP10, MPT53, and the 19 and 38 kDa lipoproteins. Epitopes have also

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Table 1. Overview of mycobacteria-specific human CD8$^+$ T cell epitopes

M. tuberculosis Ag[a] H37Rv	Amino acid sequence	HLA restriction	HLA class I-binding[b]	Tetramer staining[c]	Cyto-kines[d]	CTL activity[e]	Endo-genous Ag[f]	Anti-microbial activity[g]	Refe-rences
19 kDa LP (Rv3763)									
14–22	ILVAGLSGC	A*0201	+			–			28
88–97	VLTDGNPPEV	A*0201	+			+	+		28
101–108	GLGNVNGV	A*0201	+			–			28
38 kDa LP (Rv0934)									
45–53	TPASSPVTL	B*3501	+			+			20
175–183	LPGTAVVPL	B*3501	+		γ	+			20
292–300	TPANQAISM	B*3501	+			+			20
Ag85A (Rv3804c)									
242–250	KLIANNTRV	A*0201	+	+	γ	+	+		42
267–275	LPAKFLEGF	B*3501	+						22
Ag85B (Rv1886c)									
15–23	LMIGTAAAV	A*0201	+			+	+		6
45–53	GLPVEYLQV	A*0201	+	+	γ	+	+		16, 42
110–118	MPVGGQSSF	B*3501	+		γ	+			22
166–175	SMAGSSAMIL	A*0201	+			+	–		16
183–192	FIYAGSLSAL	A*0201	+		α γ	+	+		16
198–206	GMGPSLIGL	A*0201	+			+	–		16
239–247	KLVANNTRL	A*0201	+	+	α γ	+	+		16
264–272	IPAEFLENF	B*3501	+		γ	+			22
Ag85C (Rv0129c)									
204–212	WPTLIGLAM	B*3501	+	+	α γ	+	+		22
CFP10 (Rv3874)									
2–11	AEMKTDAATL	B*44			γ	+	+		25
85–94	RADEEQQQAL	B*14			γ	+	+		25
ESAT-6 (Rv3875)									
21–29	NVTSIHSLL	A*6802			γ	+	+		33
69–76	LQNLARTI	B*52			γ	+	+	+	24
82–90	AMASTEGNV	A*0201			γ				24
MPT53 (Rv2878c)									
134–142	VPWQPAFVF	B*3501	+		γ	+			20
PstA1 (Rv0930)									
75–83	SLYFGGICV	A*0201	+		γ	+	+	+	6
RpoB (Rv0667)									
121–129	MTYAAPLFV	A*0201	+			+			6
SPASE I (Rv2903c)									
201–210	EPYLDPATM	B*3501	+		α γ	+	+		20
ThyA (Rv2715)									
163–171	RLPLVLPAV	A*0201	+		γ	+	+	+	6

[a] Numbering is according to sequences listed in the database of predicted proteins of *M. tuberculosis* H37Rv [39].

[b] HLA class-I binding. Peptide binding has been assessed for most peptides in the table; binding is assumed in case functional data is available. Additional data on peptide binding can be found in refs. [6, 16, 22, 28].

[c] Tetramer staining. Visualization of effector CD8 T cells specific for mycobacterial peptide using PE-labeled HLA class I tetrameric complexes.

[d] Cytokines. Pro-inflammatory cytokine TNF-α and type I cytokine IFN-γ. Cytokine production determined with ELISPOT, FACS, or ELISA.

[e] CTL activity. Killing of ^{51}Cr-labeled target cells; presence of perforin or granulysin.

[f] Endogenous Ag. Killing of macrophages infected with M. tuberculosis, BCG or recombinant vaccinia viruses expressing mycobacterial antigens is taken as proof of endogenous Ag processing and presentation of the epitope.

[g] Antimicrobial activity. Ability of effector CD8 T cells to inhibit growth of intracellular mycobacteria.

been found in so-called somatic proteins, such as phosphate transport system permease protein A-1 (PstA1), RNA polymerase β-subunit protein (RpoB), signal peptidase I (SPASE I) and thymidylate synthase (ThyA) (Table 1).

Naturally processed epitopes

Various methods have been used to identify mycobacterial antigens and the respective peptides that stimulate human CD8 T cells (Fig. 1). A rather classic approach uses *in vitro* restimulation with live mycobacteria or selected antigens, and most times involves the generation of CD8 T cell clones. Epitopes are usually defined with overlapping sets of synthetic peptides[25, 42]. Although this approach will by definition reveal naturally processed epitopes, it may be expensive, time-consuming and labor intensive. The outcome with respect to HLA restriction is uncertain.

In silico prediction of CD8 T cell epitopes

Computer algorithms for HLA allele-specific binding motifs[8, 32, 35] have also been used with success to identify MHC class I-restricted epitopes in selected mycobacterial antigens[6, 16, 22, 24, 28] (Fig. 1).

The first step after *in silico* prediction usually involves screening of potential epitopes for actual binding to HLA molecules *in vitro*[9]. Predicted peptides may, however, not always show the expected binding, as requirements for auxiliary residues that enhance or interfere with binding have not been fully established[6, 16, 28]. The next step typically involves stimulation of blood samples of patients or healthy volunteers and measuring for proliferation, cytokine production or cytotoxicity. In addition, predicted binders have been screened for immunogenicity in HLA transgenic mice before confirming immune responses in humans[16].

It is possible that synthetic peptides prime reactive T cells *in vitro*, particularly after prolonged periods of restimulation with peptides and when dendritic cells are used for antigen presentation[5, 27]. However, direct *ex vivo* and short-term recall responses are generally taken as more solid evidence for priming of CD8 T cells during the natural course of infection with *M. tuberculosis*.

Despite strong binding *in vitro*, the majority of peptides fail to stimulate human CD8 T cells. The most comprehensive screening to date has been done by CHO et al.[6] who searched approximately 40 mycobacterial antigens for HLA-A*0201 peptide-binding motifs and identified 222 predicted binders (either 9 or 10 mers). After *in vitro* testing, 58 peptides showed high to intermediate affinity for HLA-A*0201. Ultimately only four new CD8 T cell epitopes (~2% of motif-bearing peptides) were discovered. Recall responses specific for ThyA (aa. 30–38) and RpoB (aa. 127–135) were observed in one of two patients tested, and for PstA1 (aa. 75–83) in two of three patients tested. No response in any of the donors tested for most of the other binding peptides suggests these are not properly processed for MHC class I presentation from their endogenous source[49]. Although the attrition rate in this study was quite high, it does suggest that in the entire mycobacterial genome we should expect to find at least a few hundred HLA-A*0201-restricted CD8 T cell epitopes.

The proportion of individuals who mount CD8 T cell responses to any given mycobacterial peptide is quite variable[6, 24, 28, 33]. For example, LALVANI et al.[24] found that only 3 out of 26 HLA-A*0201-positive donors responded to ESAT-6 (aa. 82–90), and we found that about half of HLA-B*3501-positive healthy blood donors responded to Ag85C (aa. 204–212)[22]. Heterogeneity of immune responses among individuals may limit the potential use of multi-epitope vaccines unless sufficient numbers of epitopes are included for each of the major HLA types to allow broad recognition in the population. Lack of reactive T cells may also reflect a combination of low frequencies and insufficient assay sensitivity. In addition, CD8 T cell responses may be lacking due to variable mycobacterial protein expression (either proteins are not expressed *in vivo* or only expressed during certain stages of infection), or to a "blind spot" in the T cell receptor repertoire in certain patients.

Peptides with low affinity for HLA molecules and peptides without an obvious binding motif will be largely ignored by a reverse immunogenetics approach, but could still be genuine CD8 T cell epitopes[51]. The major advantage of *in silico* predictions is that they can be extended to the entire mycobacterial genome. Therefore, in the years to come we should expect to see many more epitopes identified in previously unknown mycobacterial antigens.

Correlates of Protection – CD8 T Cells and Vaccine Issues

The current vaccine against TB consists of a series of attenuated strains of *M. bovis*, collectively termed bacillus Calmette-Guérin (BCG). BCG has minimal side effects and confers appreciable protection against extra-pulmonary TB, but its effect against pulmonary disease varies 0–80%[14]. It is widely accepted that the

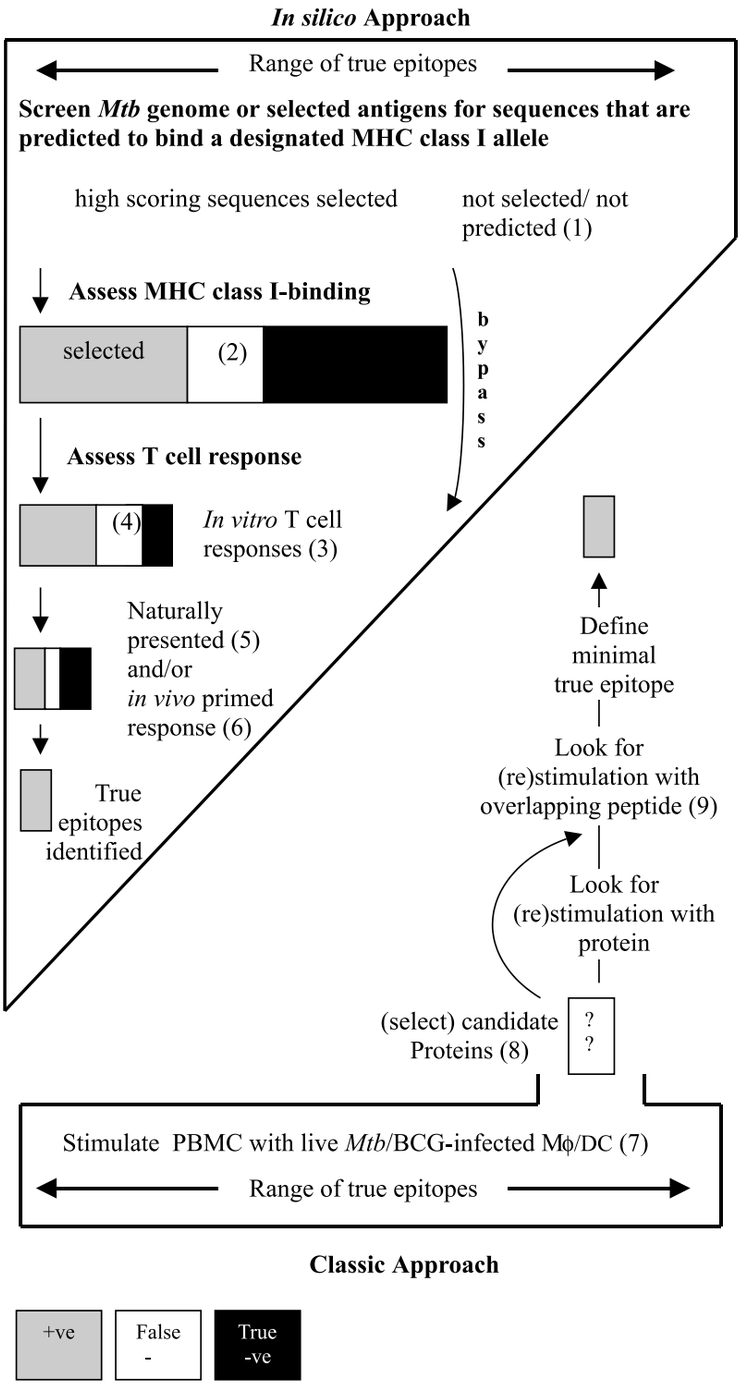

Fig. 1. Comparison of methods for identifying CD8 epitopes in *Mycobacterium tuberculosis* (*Mtb*). 1) No current evidence for 100% prediction. Many low affinity binders are not tested and cut-offs are arbitrary. 2) Low affinity binders are often not selected and may be epitopes. 3) Various studies use assays that demonstrate *in vitro* responses but provide no evidence that the protein is expressed and the peptide presented *in vivo*. 4) T cell responses to true epitopes may not be detected because of inadequate patient selection or assay sensitivity. 5) Demonstrated by the ability of the peptide to induce clones that can recognize/kill *Mtb*/BCG infected macrophage (Mϕ). 6) Measured by direct *ex vivo* or recall assays that will only stimulate primed cells. 7) Cells generated are responsive to naturally presented epitopes. 8) "Bottleneck" – testing responses to proteins (recombinant, rVV constructs etc.) and overlapping peptides can be time consuming and costly and may not reveal any candidates. This approach has only been used to test a small number of highly likely candidates. 9) Sometime stimulation of fresh peripheral blood mononuclear cells (PBMCs) from exposed individuals has been assessed *ex vivo* to provide further evidence of *in vivo* priming

majority of *M. tuberculosis*-infected individuals do not develop TB because they will mount protective immune responses. Therefore, a rational approach to designing a more effective TB vaccine would be to formulate it such that responses to vaccination mimic "natural" protective responses[1, 19]. Important issues for vaccine design include the type and dose of antigens used, the route of administration and the choice of adjuvant. However, the requirements for protection against TB in humans are still poorly characterized.

Phenotype of immune response (i.e. functional characteristics)

There is compelling evidence for an essential role of type I cytokines in protective immunity to intracellular bacteria (reviewed in ref.[30]). Mycobacteria-specific CD8 T cells have been reported to produce type I cytokines (IFN-γ and TNF-α) and to release cytolytic molecules (e.g. granzymes, perforin and granulysin) upon antigen recognition. CD8 T cells are capable of cytolytic activity (as demonstrated by their ability to lyse mycobacteria-infected macrophages) and of indirect bactericidal activity (as demonstrated by the ability to inhibit intracellular growth of mycobacteria) (Table 1; reviewed in refs.[15, 43]). Although these features are not entirely exclusive to CD8 T cells, the collective properties are thought to play a major role in protection against TB.

Magnitude of immune response (i.e. frequencies)

It is expected that the numbers of mycobacteria-specific CD8 T cells are higher in situations where people have been exposed and successfully contained the infection. WILKINSON et al.[54] reported seven exposed healthy contacts with CD8 T cells specific for the 38 kDa antigen, and seven untreated TB patients with very low levels of 38 kDa Ag-specific IFN-γ-secreting cells. Similarly, PATHAN et al.[33] reported one exposed healthy household contact with an unusually high frequency of IFN-γ-secreting CD8 T cells specific for ESAT-6. In contrast, TB patients appear to have much lower frequencies of mycobacteria-specific CD8 T cells[6, 44]. The data suggest that strong CD8 T cell responses contribute to the control of *M. tuberculosis* infection in humans, although still only limited numbers of patients have been reported, mostly from cross-sectional studies. Lower frequencies in TB patients could, on the other hand, reflect reactive CD8 T cells homing to affected tissues rather than remaining in the peripheral circulation[13, 36, 38].

In BCG-vaccinated healthy donors, virtually no specific staining with HLA class I tetramers is observed in unstimulated PBMC directly *ex vivo*[16, 22, 42]. Using the more sensitive ELISPOT technique for detection of IFN-γ spot-forming cells, significant frequencies of mycobacteria-specific CD8 T cells are readily detected. Low responses in some BCG-vaccinated donors may be related to when BCG was given. Meta-analysis of randomized controlled trials has indicated that protection by BCG wanes with time. Ten to 15 years after vaccination there appears to be little epidemiological evidence that BCG provides protection against TB[47]).

Heterologous immunity

Many studies have shown that mycobacteria can induce cross-protection against diseases caused by related species. As discussed, BCG vaccination provides variable protection against TB, but, similarly, against diseases caused by *M. leprae*, *M. avium*-intracellulare and *M. ulcerans*[14].

The protective efficacy of BCG appears consistently lower in countries with a higher suspected prevalence of environmental mycobacteria. Palmer's classic experiments with guinea pigs already suggested that exposure to environmental mycobacteria protects against subsequent challenge with *M. tuberculosis*[31]. However, pre-existing heterologous immunity may also limit the multiplication of BCG *in vivo* that is needed to acquire sufficient protection against TB. It follows then that BCG could do little to improve upon such naturally acquired immunity[1]. T cell responses directed against highly conserved epitopes could play a major role in limiting the effects of BCG. Conversely, boosting of immune responses against conserved sequences may in fact help to achieve protection against TB. In the current list of human CD8 T cell epitopes only two epitopes are completely conserved in the five mycobacterial genomes analyzed to date (Table 2). A more comprehensive search for conserved mycobacterial sequences and T cell epitopes is required in order to further understand cross-protection induced by BCG vaccination and by exposure to environmental mycobacteria.

Environmental mycobacteria that share antigenic determinants (i.e. highly related but not identical), may prime the immune system in such a way that, upon BCG vaccination or exposure to *M. tuberculosis*, reactive T cells are antagonized by altered peptide ligands. This phenomenon of "original antigenic sin" was first described for the antibody responses to influenza virus, but also applies to T cell responses in persistent infec-

Table 2. Sequence variation in mycobacterial human CD8 T cell epitopes

Mycobacterium	**19 kDa LP (Rv3763)**			**38 kDa LP (Rv0934)**			**Ag85A (Rv3804c)**		**Ag85C (Rv0129c)**
TB H37Rv	VLTDGNPPEV	GLGNVNGV	KITGTATGV	TPASSPVTL	LPGTAVVPL	TPANQAISM	KLIANNTRV	LPAKFLEGF	WPTLIGLAM
TB CDC 1551	----------	--------	---------	---------	---------	---------	---------	---------	---------
bovis AF2122/97	----------	--------	---------	---------	---------	---------	---------	---------	---------
leprae TN	**********	---TID--	Q-----S--	------G-T	----T----	*********	R-V-----I	----L---L	---M-----
avium	--S-----Q-	--------	---------	*********	*********	*********	--V-----I	---------	---------
smegmatis	**********	A---ID--	*********	*********	*********	*********	R-V-----I	---------	--M------

Mycobacterium	**Ag85B (Rv1886c)**								**MPT53 (Rv2878c)**
TB H37Rv	LMIGTAAAV	GLPVEYLQV	MPVGGQSSF	SMAGSSAMIL	FIYAGSLSAL	GMGPSLIGL	KLVANNTRL	IPAEFLENF	VPWQPAFVF
TB CDC 1551	---------	---------	---------	----------	----------	---------	---------	---------	---------
bovis AF2122/97	---------	---------	---------	----------	----------	---------	---------	---------	---------
leprae TN	--L---GS-	---------	---------	-------L--	----------	A--------	R-------I	V--------	-----PK--
avium	-LV-A----	---------	---------	-------L--	----------	---------	--------I	M--------	------Y--
smegmatis	*********	-------M-	---------	-----A-L--	------M-GF	-WW-M----	R-------I	L--K---G-	*********

Mycobacterium	**CFP10 (Rv3874)**		**ESAT-6(Rv3875)**			**PstA1 (Rv0930)**	**RpoB (Rv0667)**	**SPASE I (Rv2903c)**	**ThyA (Rv2715)**
TB H37Rv	AEMKTDAATL	ADEEQQQAL	NVTSIHSLL	LQNLARTI	AMASTEGNV	SLYFGGICV	MTYAAPLFV	EPYLDPATM	RLPLVLPAV
TB CDC 1551	----------	---------	---------	--------	---------	---------	---------	---------	---------
bovis AF2122/97	----------	---------	---------	--------	---------	---------	---------	---------	---------
leprae TN	---I-E--I-	*********	----L--IT	********	*********	*********	---------	----R-V--	*********
avium	**********	*********	*********	********	*********	*********	---------	-----RN--	---------
smegmatis	-A-N----V-	*********	*********	-----Q--	*********	*********	---------	------T--	-------SL

The genome sequences of *M. avium* and *M. smegmatis* were not fully finished at the time of preparing this table.
Alignment of the amino acid sequences (one-letter code) of currently known human CD8 T cell epitopes.
Sequence information was obtained from The Sanger Centre[39] and The Institute for Genomic Research[48].
The sequence of *M. tuberculosis* H37Rv is shown for comparison. Dashes indicate identical amino acid residues; asterisks mean there were less than 6 identical residues.

tions with hepatitis B virus or HIV-1 and in parasitic infections such as malaria[4, 23, 34]. A similar mechanism may play a role in mycobacterial infections. From the reported CD8 T cell epitopes it is clear that environmental mycobacteria indeed have sequences very similar to sequences found in BCG and *M. tuberculosis* (Table 2). These variant peptides may well bind to HLA, but at the same time could affect signaling through the T cell receptor and antagonize reactive T cells. This scenario would argue for the use of truly unique *M. tuberculosis* antigens or absolutely conserved sequences as post-exposure vaccines for TB. The former is probably preferable, as exposure to environmental mycobacteria is expectedly heterogeneous and uncontrolled.

An alternative explanation for the variable results with BCG is that environmental mycobacteria may polarise the immune system towards a type-2 mode, resulting in less efficacious immune responses after BCG vaccination or exposure to *M. tuberculosis*[37]. ROOK et al.[37] have shown in mice that prior exposure to some environmental mycobacteria enhances protective efficacy of BCG, whereas other species appear to reduce it. Two types of immune response were induced by environmental mycobacteria and this correlated with their effect on the efficacy of BCG[37]. In humans it is unclear which environmental mycobacteria induce protection against TB and which achieve the opposite. It is of interest that BCG, when given at birth, has been shown to induce a significant type-1 response[26]. Early priming of the immune system with the appropriate mycobacterial species or subunit antigens may indeed favor the development of protective immunity to TB later in life.

Subunit vaccine candidates

Most of the known human CD8 T cell epitopes are found in secreted mycobacterial antigens. The significance of these findings is inferred from observations in animal models where killed, whole mycobacteria impart inferior protection. HORWITZ et al.[18] have shown in guinea pigs that purified secreted antigens can induce strong cell-mediated immune responses and give substantial protection against aerosol challenge with virulent tubercle bacilli.

Dominant CD8 T cell responses against the major secreted antigens of the Ag85 complex have been observed both in healthy BCG-vaccinated donors and in TB patients[22, 46, 44]. These antigens are currently among the most promising for novel subunit TB vaccines. In mice it has been shown that vaccination with plasmid DNA constructs encoding Ag85A conferred significant protection against challenge with live *M. tuberculosis*. Substantial cell-mediated immune responses were induced after vaccination. When reactive CD8 T cells were analyzed in more detail, it was found that the epitope repertoire after plasmid DNA vaccination was broader than after live infection with *M. tuberculosis*[10]. In addition, OLSEN et al.[29] succeeded in protecting mice by immunization with a subdominant epitope from ESAT-6 to which only negligible responses were mounted during natural infection. Collectively, these observations suggest that natural immunity to mycobacteria can be improved or at least supplemented by vaccination.

CD8 T cell epitopes have also been found in somatic mycobacterial proteins, suggesting that the actual panel for subunit TB vaccines may not necessarily be restricted to secreted antigens[6, 20]. In addition, several proteins from the PE and PPE families have been found to induce both CD4 and CD8 T cell responses. While the cellular location and function are unknown, these antigens are interesting because they comprise about 10% of the *M. tuberculosis* genome[11, 41]. Also of interest to the development of subunit vaccines are T cell epitopes in proteins that are upregulated in mycobacteria after they infect macrophages and in mycobacterial antigens that are selectively expressed during clinical latency.

Animal models for TB

The current practice is to evaluate experimental vaccines in animal challenge studies. However, at present there is no suitable animal model that mimics the full spectrum of clinical latency and pathology of *M. tuberculosis* infection in humans. There are several mouse, guinea pig and primate models of TB, each of which have interesting features for testing certain aspects of immunity to mycobacteria. For example, HLA-B*35 epitopes (part of the HLA-B7 super-family) may be evaluated directly in BALB/c mice for H-2L^d-restricted CD8 T cell responses because of the similarity of MHC class I-binding requirements[35]. One of the peptides identified after vaccination of BALB/c mice with Ag85-plasmid DNA[10] was found to be immunogenic in HLA--B*35-positive individuals[22]. Of particular value are animal models in which human MHC molecules are expressed as transgenes. GELUK et al.[16] used HLA-A*0201 transgenic mice to successfully identify human CD8 T cell epitopes. Such mouse models could be exploited further for novel vaccination strategies and challenge studies.

Novel Tools for Improved Diagnosis of Infection and Disease

The current spectrum of TB diagnostics include clinical examination, chest radiography, combined with tuberculin-based skin test, direct microscopic examination of sputum and culture of mycobacteria. The poor specificity and sensitivity of these diagnostic tools generally hampers rapid diagnosis of TB. Furthermore, diagnosis of pediatric and extra-pulmonary TB remains notoriously difficult. Another problematic area is the assessment of latent or subclinical *M. tuberculosis* infection. Immune-based tests using recall responses to specific mycobacterial antigens may detect whether the immune system has been sensitized by *M. tuberculosis* or other mycobacteria and could possibly assist in the diagnosis of TB[2, 12].

Immunodiagnosis of M. tuberculosis infection

Several mycobacterial genome sequences are now available[39, 48.] This will allow the identification of specific antigens or sequences that could be used as diagnostic reagents. Whole genome DNA microarray technology has revealed 16 so-called RD-regions with at least 129 ORFs that are present in *M. tuberculosis* but have been deleted in *M. bovis* BCG[3]. Immune responses to ESAT-6 and CFP10, proteins encoded in RD1, may possibly be used to differentiate between *M. tuberculosis* infection and BCG vaccination. CD8 T cell responses against ESAT-6 and CFP10 have been reported both in TB patients and exposed healthy individuals[24, 25, 33]. Smith et al.[44] observed significant CD8 T cell responses to ESAT-6 in pulmonary TB patients reminiscent of active *M. tuberculosis* infection, whereas no responses to ESAT-6 could be detected in healthy BCG-vaccinated donors.

The use of ESAT-6 and CFP10 to differentiate between *M. tuberculosis* complex species and environmental mycobacteria may be limited, as they are also expressed in *M. kansasii, M. marinum, M. szulgai*, and *M. gastri*, as well as in virulent *M. bovis* but not in BCG strains[2, 7]. In addition, it appears that *M. smegmatis* contains an ORF with 71% sequence identity to ESAT-6 of *M. tuberculosis*. Consequently, we found a sequence in *M. smegmatis* that appears nearly identical (substitution of R to Q at position 6) to the reported HLA-B*52-restricted epitope in ESAT-6[24] (Table 2). Whether this variant sequence will induce heterologous reactivity or antagonize the immune response to *M. tuberculosis* remains to be tested[4, 23, 34].

Amino acid sequences of human CD8 T cell epitopes identified so far appear identical in at least 2 strains of *M. tuberculosis* and one strain of *M. bovis* (Table 2). Except for epitopes found in ESAT-6 and CFP10, all other epitopes are also present in BCG vaccine strains, and are thus not suitable to differentiate between *M. tuberculosis* infection and BCG vaccination. Moreover, about one third of the sequences are also found in *M. avium*. The specificity of immunodiagnostic tests based on mycobacterial antigens or epitopes will vary with the prevalence of environmental mycobacteria in the population under study. With the available sequence information it should be feasible to identify strain-specific T cell epitopes in a more comprehensive way, by aligning whole genome sequences and searching for unique sequences. Novel immunodiagnostic tools may have improved specificity over current tests, but sensitivity will probably remain an issue in immune-compromised individuals, such as HIV-1--infected individuals and advanced TB patients.

Prognostic markers of disease

The phenotype of reactive CD8 T cells during particular pre-clinical conditions could possibly be explored to develop prognostic tools for predicting disease. Circulating T lymphocytes from TB patients appear to produce significant amounts of IL-4[40, 50]. Remarkably, the expression of IL-4 is particularly elevated in CD8 T cells of TB patients with pulmonary cavities. We have also found increased IL-4 production by CD8 T cells from TB patients compared to BCG--vaccinated controls when cells were stimulated with live BCG or *M. tuberculosis*[45]. This suggests that during active disease there is a more polarized type-2 immune response, which may be less effective in controlling the infection or may even be detrimental to the host.

The issue to be addressed is whether a particular phenotype of reactive T cells can be detected in an early stage of *M. tuberculosis* infection, preferably before the onset of disease. In addition, persistence of a particular phenotype during chemotherapy may provide an early indication of possible treatment failure or development of multi-drug-resistant TB. IFN-γ and IL-4 have been studied as classical representatives of type-1 and type-2 immune responses, respectively. With the introduction of DNA microarray technology we should expect to see more comprehensive phenotyping of reactive T cells. The combination with long-term clinical follow-up of exposed individuals and TB patients will allow us to identify markers associated with disease and possibly to develop more sophisticated prognostic markers.

Concluding Remarks

There is increasing evidence that CD8 T cells are important for the control of *M. tuberculosis* infection. Emerging technologies such as HLA-tetramers and DNA microarrays in combination with key mycobacterial genome sequences should foster major breakthroughs in TB immunology in the near future. Areas and issues to be addressed include the following:

- Continue to expand our knowledge of mycobacterial antigens and T cell epitopes that are targeted by the immune system during various stages of *M. tuberculosis* infection and disease. This will provide crucial information for the development of potential subunit vaccines, as well as for the development of immunodiagnostic tools.
- Long-term follow-up of exposed contacts of TB patients in order to correlate features of cellular immunity with clinical outcomes. This will yield important information for the development of surrogate markers that are needed to provide early indications of potential vaccine efficacy.
- Launching of an interactive Internet website where TB immunology data can be accessed similarly to the HIV Molecular Immunology Database[17] and linked to sites with mycobacterial genome sequences[39, 48] and the relevant bioinformatics tools[8, 32, 35].

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