Supplementary Box 1 | **MHC-E-restricted T cells elicited by pathogens**

Mycobacterium tuberculosis

Mtb-specific T cell clones derived from a latently infected individual recognized *Mtb*-antigen presented by HLA-E[1]. Using mass-spectrometry (MS), some Mtb-derived peptides were identified that stimulate T cells[2]. These non-canonical HLA-E-binding Mtb peptides were shown to be processed in late endosomes and in *Mtb*-containing phagosomes[3,4] (**Fig. 3**). HLA-E-binding peptides within the *Mtb* genome were also identified using *in silico* analysis followed by peptide binding studies[5-7] and some of these peptides elicited proliferation of T cells obtained from *Mtb*-infected patients[6]. $CD8^+$ T cell clones generated from these individuals were able to lyse Mtb-infected cells[8]. HLA-E–peptide-specific T cells were also identified using tetramers in humans[7-11] as well as in rhesus and cynomolgus macaques experimentally infected with *Mtb*[7,9]. Unexpectedly, HLA-E tetramers stained not only $CD8^+$, but also $CD4^+$, T cells in both humans and non-human primates (NHPs), including T cells with Th2 type cytokine expression and regulatory phenotypes[9]. The frequencies of tetramer-reactive $CD4^+$ and $CD8^+$ T cells did not change in humans and NHPs upon BCG vaccination but increased in the lungs of unvaccinated NHPs upon *Mtb* challenge[9]. TCR-sequencing revealed a highly diverse repertoire of clonotypes within and between individuals[12]. These studies revealed that HLA-E-restricted *Mtb*-specific T cells are more similar to classically restricted T cells with respect to TCR usage compared with other non-classically restricted αβ TCR-expressing T cells, such as CD1-restricted NKT cells or MR1-restricted invariant T cells, that are characterized by limited TCR variability[13]. However, it is not yet known what role, if any, these T cells play in controlling *Mtb* infection. Recent advances in eliciting MHC-E-restricted T cells might be able to answer these questions. Moreover, it was recently demonstrated that a bispecific protein consisting of an affinity-enhanced Mtb44-specific TCR and an anti-CD3 antibody (ImmTAB) enables polyclonal T cell populations to recognize infected cells and reduce intracellular *Mtb* infection[14] (**Fig. 2**). Thus, even if naturally elicited HLA-E-restricted T cells are not involved in controlling natural *Mtb* infection, *Mtb* might still be susceptible to HLA-E-targeting immunotherapeutics or vaccines.

Salmonella

$CD8^+$ T cells from a subset of individuals vaccinated with *Salmonella typhi* Ty21a strain mediated HLA-E-dependent killing of *S. typhi*-infected cells[15,16]. Moreover, T cells were stimulated with peptides from the *S. typhi* GroEL sequence based on known HLA-E motifs in the vaccine[15]. HLA-E-restricted *S. typhi*-specific T cells displayed an effector memory phenotype[17] and their baseline presence was associated with delayed disease in a human *S. typhi* challenge model[18]. However, another study found that high levels of both conventional and unconventional T cells at baseline were associated with increased risk of disease at higher doses of challenge[19].

HIV

HLA-E escapes the downregulation of classical HLA-Ia by the HIV Nef and Vpu proteins[20,21], and HLA-E was shown to be upregulated in HIV-infected $CD4^+$ T cells[22]. However, Nef from some primary HIV-1 strains reduced HLA-E surface expression, suggesting a range of HLA-E levels on virally infected cells[23]. HLA-E-restricted T cells are rarely observed in HIV-infected individuals and the HIV proteome does not contain many peptides binding with high affinity to HLA-E[24,25]. However, using disulfide-trapped or UV-exchanged tetramers containing the Gag peptide RL9 (RMYSPVSIL) (which binds with moderate strength to HLA-E[25-27]), T cell clones from HIV-negative donors could be isolated. T cells expressing these TCRs controlled HIV in viral inhibition assays in an HLA-E-dependent, classical HLA-Ia-independent manner[28]. Furthermore, the Gag peptides KL9 (KALGPAATL) and KF11 (KAFSPEVIPMF) are presented by both classical HLA-Ia (HLA-B57) and HLA-E[29]. In HIV-infected individuals both unique HLA-E-restricted responses and dually restricted responses were observed. Moreover, KF11-specific effector cells expanded *in vitro* responded to HIV-infected target cells expressing HLA-E. Both studies thus demonstrated that endogenously processed and weak binding peptides can be loaded onto HLA-E in HIV-infected cells and targeted by $CD8^+$ T cells.

In contrast to T cells, MS analysis of HLA-E-bound peptides from HIV-infected cells failed to identify any HIV peptides[25] likely because immunopeptidomics workflows do not

efficiently capture peptides that bind HLA-E with short half-lives[27,30]. Detailed biophysical analysis of HIV peptide interactions with HLA-E by small-angle X-ray scatter (SAXS) showed that HLA-E-bound HIV-derived peptides produce larger SAXS dimension compared with VL9 peptides or Mtb44, consistent with unstable complex formation[26]. Structural analysis of RL9 peptide bound to HLA-E revealed that the reason for this lower overall stability is a limited interaction of RL9 with the peptide-binding groove[24,27]. Such unstable non-VL9 peptides thus introduce an alternative surface recognition landscape that allows TCRs to discriminate these complexes from HLA-E–VL9[31]. The low stability of HIV peptide–HLA-E interaction renders the development of TCR-based immunotherapeutics challenging. For instance, a soluble affinity-enhanced HLA-E–RL9-specific TCR fused to an anti-CD3 scFv was only able to redirect T cells to target cells loaded with exogenous peptide, but not exogenously expressed RL9 minigene, Gag protein or HIV-infected cells[25].

SARS-Cov-2

HLA-E is upregulated whereas classical HLA-Ia is downregulated in SARS-CoV-2-infected cells[32]. The SARS-CoV-2 genome contains several HLA-E-binding peptides[33,32] and some of these peptides were recognized HLA-E-dependent $CD8^+$ T cells from Ad5/CoV-2-vaccinated individuals[33] or by individuals recovering from COVID-19[32]. Interestingly, the frequency of HLA-E-restricted T cells correlated with disease severity[32]. Tetramer-sorted and TCR-transfected, $CD8^+$ T cells inhibited SARS-CoV-2 replication[32]. Thus, HLA-E-restricted T cells might contribute to SARS-Cov-2 control, suggesting that ‘off-the shelf’ TCR-based therapies might control this infection[34].

Epstein–Barr virus

VL9–HLA-E-reactive $CD8^+$ T cells also recognized the peptide SQAPLPCVL in the Epstein–Barr virus (EBV) protein BZLF1[35], and HLA-E–SQAPLPCVL tetramers stain $CD8^+$ T cells in multiple sclerosis, a disease since linked with EBV, but not in healthy controls[36]. HLA-E–SQAPLPCVL-specific $CD8^+$ T cells also inhibited EBV spread *in vitro*[37]. Viral inhibition was more efficient for HLA-E*01:03 compared with HLA-E*01:01, and the

HLA-E*01:03/01:03 genotype was protective against infectious mononucleosis[37]. Thus, HLA-E-restricted T cells seem to play a role in controlling acute EBV infection.

Influenza virus

The cryptic epitope M-SL9 mapping to an unannotated alternative reading frame product of the influenza matrix gene segment of influenza virus was presented by Qa-1 and elicited a strong Qa-1-restricted $CD8^+$ T cell response in infected mice[38]. M-SL9–Qa-1-specific T cells were elicited by immunization with mRNA encoding a C-terminal fusion of M-SL9 with the lymphocytic choriomeningitis virus glycoprotein. Vaccine-induced T cells demonstrated *in vivo* cytolytic activity against M-SL9 peptide-loaded splenocytes. However, protection against influenza challenge was not evaluated.

Hepatitis C virus

Hepatitis C virus (HCV) upregulates HLA-E, and a screen of HCV core peptides binding to HLA-E yielded the 10mer HCV core peptide YLLPRRGPRL, a known HLA-A2 epitope[39]. This highly conserved peptide is recognized via HLA-E by circulating $CD8^+$ T cells from HCV-infected individuals[40]. The best HLA-E-restricted responses were recorded in patients homozygous for HLA-E *01:01. Moreover, these individuals responded better to anti-HCV therapy[41,42] and were under-represented in chronic infections with HCV genotypes 2 and 3, consistent with a protective effect of HLA-E-restricted $CD8^+$ T cells.

Hepatitis B virus

Upon *in silico* analysis of hepatitis B virus (HBV) envelope proteins, 70 peptides were screened for binding to HLA-E[43]. 6 peptides tested showed measurable binding, with ILSPFLPLL displaying the highest affinity. Using page display, a TCR specific for this peptide and two variants was affinity enhanced. The crystal structure of this TCR revealed a canonical interaction of the α and β chains with the HLA-E helix 2 and 1, respectively, whereas the CDR3 interacted with each of the three variant peptides in the peptide-binding groove. A bispecific fusion with anti-CD3 antibodies redirected T cells to HLA-E-expressing HBV-infected cells. The bispecific molecule mediated lysis of a liver cell line

expressing the HBV S antigen from integrated HBV DNA. Interestingly, MS analysis of the HLA-E peptidome from these cells did not identify the HBV peptide, consistent with T cell assays being more sensitive than immunopeptidomics. HBV replication in HLA-E-expressing HepG2 cells transfected with replication-competent HBV cDNA was reduced by the bispecific. HBV peptide-specific $CD8^+$ T cells were detected by HLA-E–HBV dextramers in both chronic HBV carriers and one HBV naive volunteer, after two rounds of *in vitro* stimulation with artificial antigen-presenting cells. Since HLA-E is expressed in HBV-infected livers, this study suggests that TCR-based immunotherapeutics could be used against HBV.

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