Systematic analysis of T cell responses specific to the Epstein-Barr virus proteome using ATLAS™

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ABSTRACT

Epstein-Barr virus (EBV) is a human herpesvirus infecting 95% of the world population. Primary infection can cause acute infectious mononucleosis (AIM), mainly when EBV is acquired later in life. In addition, EBV establishes latency and has been associated with several cancers. To date, efforts to develop vaccines for prevention of infection or disease by induction of g350-specific neutralizing antibody responses have failed. To improve antigen selection for future vaccines, we utilized ATLAS™, a screening platform that can characterize the specificity of CD4+ and CD8+ T cell recall responses to the entire EBV proteome without bias of immunodominance.

Firstly, we measured antigen-specific T cell activation in 15 AIM patients longitudinally during and 6 weeks after the asymptomatic disease phase. For both T cell subsets, the breadth of response significantly decreased with resolution of disease. Likely in parallel to the contraction of the CD8+ T cell compartment associated with AIM clearance. Notably, we detected a substantial overlap in antigen specificity between T cell responses in samples collected during AIM and in convalescence.

Secondly, we identified EBV antigens that elicit T cell responses in both seropositive and seronegative but possibly exposed adolescents. Interim analysis revealed that ATLAS™ not only confirmed several previously described CD4+ and CD8+ T cell targets in latently infected individuals, but also identified novel T cell antigens independently of serostatus. Ongoing comparative analyses will focus on differences in the memory T cell repertoire between cohorts and on antigens overrepresented in exposed seronegative individuals that may be protective and therefore of value for vaccine development.

INTRODUCTION

Epstein-Barr virus (EBV) is a γ-herpesvirus, infecting >95% of the population worldwide and establishing lifelong latency mainly in B cells and epithelial cells. The majority of primary infections occur during childhood with minimal symptoms, but infection later in life can be associated with acute infectious mononucleosis (AIM). EBV is also associated with several types of cancer in both immunocompetent and immunocompromised individuals, totaling 200,000 cancer cases per year worldwide and 1% of all malignancies.

To date, no prophylactic vaccine capable of preventing seroconversion has been approved. However, several therapeutic approaches using or inducing EBV-specific T cells are under development. In EBV-positive tumors, only a limited number of latent EBV proteins are expressed and targeting very few antigens increases the risk of tumor escape from immunological pressure. Nevertheless, adaptive T cell therapies in patients with post-transplant lymphoproliferative disease after hematopoietic stem cell transplants are highly effective.

T cell responses to latent and a selection of lytic antigens have been well characterized in EBV-associated cancer patients and acute infection, respectively. Responses to other EBV proteins are less understood. Here, we are using an unbiased screening approach to characterize responses of both T cell subsets to the entire EBV proteome in infected and uninfected individuals. By identifying antigens that are differentially responded to dependent on EBV serostatus, T cell subset, and during and after resolution of AIM, we aim to identify potential targets for vaccine development in both the infectious diseases and oncology space.

STUDY DESIGN

Table 1: Whole blood was collected from adolescents with different EBV infection and disease status, and CD4+ and CD8+ T cell responses were characterized separately against the EBV proteome. Cohort sizes and screened T cell subsets are listed. Cohort assignments were serologically confirmed using VCA-IGM, VCA-IgG, and EBNA-IgG ELISAs.

<table>
<thead>
<tr>
<th>Sample Information</th>
<th>EBV Proteome Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort definition</td>
<td>No. of AIM/seropositive samples screened</td>
</tr>
<tr>
<td>GEA AIM patients</td>
<td>17</td>
</tr>
<tr>
<td>GSP sero-negative</td>
<td>25</td>
</tr>
<tr>
<td>GSP sero-positive</td>
<td>22</td>
</tr>
</tbody>
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96 full-length EBV proteins were cloned into the ATLAS™ expression vector and sequence- and expression-verified. 6 proteins had to be cloned as overlapping fragments to ensure sufficient expression levels. Clones were expressed in 2 E. coli strains: (i) coexpressing hystisynovin D (cLLO) to facilitate MHC class I presentation by releasing the contents of the phagolysosome into the cytoplasm, and (ii) without cLLO facilitating MHC class II presentation through the conventional endocytic processing route.

CONCLUSIONS

- By screening against the entire EBV proteome in an unbiased manner, ATLAS™ was able to identify both known and novel T cell responses to EBV antigens.
- Expectedly, CD8+ T cell responses were broader in AIM patients and this bias was much less pronounced for CD4+ T cells. The breadth of response declined with resolution of disease, and in parallel with the contraction of the CD8+ T cell compartment in this phase.
- Overall, responses in asymptomatic seropositive and seronegative individuals were more similar to each other than response profiles from AIM patients at.
- ATLAS™ identified a considerable number of antigens that suppress Th1 cytokine production, including two antigens with suppressive responses in >85% of asymptomatic seropositive and seronegative donors. Potential mechanisms for this suppression are under investigation.
- Several antigens were uniquely responded to in seronegative individuals, albeit with low frequencies within this cohort. Assuming likely exposure to EBV, these antigens may represent potentially protective antigens relevant for future vaccine development.
- In seropositive donors, recall responses could be detected not only to classic latent antigens, but also to lytic EBV proteins that have not been commonly mapped before. These antigens may be of interest for development of immunotherapies of EBV-associated diseases.

REFERENCES