REA.05 - High density peptide microarrays – an essential tool for fast development of serological assays: example of Hepatitis E Virus

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Introduction:

Serological diagnosis of viral diseases often remains challenging either due to cross-reactivity towards protein antigens, heterogeneous immune responses or poorly defined antigenic properties of proteins used in serological assays. A single or multiple well-characterized epitopes can present a much better specificity. However, it is more expensive and time-consuming to develop such assays. We present here a platform for the fast and inexpensive identification of multiple peptide epitopes for the development of highly specific serological assays. As an example, we chose to identify Hepatitis E Virus (HEV) epitopes. HEV is increasingly recognized as an emerging pathogen which can cause fatal disease in pregnant woman and immune-compromised patients, such as organ transplant recipients. While acute stages of the disease can be diagnosed using nucleic acid amplification techniques, the overall burden of the disease in the general population can only be estimated using state of the art serological assays.

Objective:

HEV serological assays of different manufacturers vary considerable due to differences in the antigen content. Serological assays based on carefully selected peptides are ideally suited to overcome these problems. The objective of this study is to show that high density peptide microarrays can be used as a tool to carefully select highly specific and sensitive epitopes in HEV in a very inexpensive and fast way.

Methodology:

We translated the whole proteomes of prototype strains of all human pathogenic genotypes of HEV into linear or cyclic peptides. In total, we synthesized 5.426 peptides on a single microarray. We incubated the microarrays with sera of patients infected with HEV and compared their IgG binding profile to samples of non-infected patients in order to identify HEV specific antibodies.

Results:

We analyzed 6 IgG-positive and 3 IgG-negative sera both using linear and cyclic peptides. Overall, we found a very heterogenic immune response towards HEV proteomes. We identified clear differences between seropositive and seronegative individuals in the overall humoral response pattern, as well as at a single peptide level. Discriminatory immunogenic regions were specially identified in the N-terminal part of the capsid protein and at the C-terminal end of ORF3. In these regions, a couple of peptides could clearly discriminate between HEV positive and negative patients. In total, these results were obtained in only 4 weeks: 1 day for HEV peptide library design, 3 weeks for microarrays production, 4 days for immunoassay and data analysis.

Conclusion:

Although the results shown here do not present a ready-to-use serological test, our data show that high density peptide microarrays can identify disease specific epitopes in a very fast and inexpensive way. Our platform enables the discovery of novel linear and conformational epitopes that can lead to the development of innovative and multiplexed serological assays with a higher sensitivity and specificity.

Keywords: Development of serological tests, High density peptide microarrays; Viral proteome