Karolinska Institutet

Institutionen för medicin, Huddinge

Elicitation and enhancement of T and B cell responses

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Lecture Hall CMB, Berzelius väg 21, Karolinska Institutet, Solna

Fredagen den 26 oktober 2012, kl 09.00

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Stockholm 2012

ABSTRACT

The Major histocompatibility complex class I (MHC-I) has been characterized in such great depth that a number of its key properties are well understood and part of its behavior can even be predicted. It is therefore intriguing that the impact of small epitope modifications on immunogenicity and the elicitation of T-cell repertoires remain often unpredictable and full of surprises.

Substitution of the secondary anchor residue at peptide position 3 from a serine to a proline (p3P) significantly increased the stabilization capacity and immunogenicity of the melanoma-associated H-2D^b (D^b)-restricted epitope gp100 (EGS). Despite this strong enhancement the conformation of the modified epitope (EGP) was not altered and vaccination with EGP generated T-cell responses that recognized cells expressing EGS with high functional avidity. Based on these promising results, the p3P modification was applied to the highly immunodominant *Lymphocytic Choriomeningitis Virus* epitope gp33 and the associated escape variants Y4F and Y4A. As for gp100, p3P was found to increase the MHC stabilization capacity and immunogenicity of the modified epitopes V3P, PA and PF, while not altering their structures. Accordingly, T-cell responses were cross-reactive between native and p3P enhanced epitopes and, when used for vaccination of C57BL/6 mice, PF elicited a focused T-cell response against D^b/Y4F.

In parallel, surface plasmon resonance (SPR) measurements revealed that p3P did not only enhance MHC stabilization capacity but also directly increased the affinity of the cognate T-cell receptors (TCRs). To fully characterize the molecular details underlying these two enhancing effects, the thermostability, TCR binding and molecular dynamics (MD) of D^b/EGP were measured in comparison with D^b/EGS. Furthermore, the contribution of Y159, a highly conserved tyrosine that is structurally juxtaposed to p3P, was assessed using a set of soluble D^b-Y159 variants. In conclusion, these measurements clearly demonstrated that specific interactions of p3P with the aromatic ring of Y159 are responsible for the significantly increased MHC stabilization capacity. Surprisingly, the enhanced TCR binding was found to be entirely independent of Y159, suggesting a direct contribution of the buried proline residue to TCR binding. These findings underscore the potential to enhance MHC-I-restricted epitopes at secondary anchor residues, while specifically indicating that proline can directly increase TCR affinity, which could not have been anticipated from our current understanding of the factors shaping TCR recognition.

Not entirely different from T-cell elicitation, the induction of broadly neutralizing antibodies against the *Human Immunodeficiency Virus* type 1 (HIV-1) is to date still an elusive goal despite extensive characterization of the respective antibody-epitope interactions. One of the central challenges is that the virus is highly adapted to immune pressure and the most relevant antibody epitopes on the HIV envelope proteins (Env) are the least immunogenic. Therefore, a highly heterologous prime-boost vaccination strategy was designed in which priming of rabbits with HIV-1 *env* plasmids was followed by a recombinant *Simian Immunodeficiency Virus* (SIV) Env boost. While the SIV Env trimers were inherently favorable because of their higher stability, the approach was specifically chosen to preferentially boost antibody responses against the few sites that are conserved in HIV and SIV Env. The described approach was generally validated and warrants future investigations as it lead to the elicitation of potent neutralizing antibodies even though it remains to be fully established if the highly heterologous nature of the prime boost strategy was solely responsible.

In summary, the studies presented in this thesis provide the structural and functional platform for a novel and intriguing MHC-I peptide enhancement. Additionally, heterologous immunizations of rabbits offer a promising addition to existing vaccination strategies against HIV.

ISBN 978-91-7457-910-9