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Dissertations-Kurzfassung

Identification of HLA-DRB1*0301-restricted T Cell Epitopes from Melanoma Antigen Tyrosinase-related Protein-2 and its Bacterial Vector *Listeria monocytogenes*

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$CD4^+$ T helper (Th) cells play a critical role in the induction and maintenance of cytotoxic anti-tumor immunity. Great efforts are now being made to mobilize such T cells against tumors in patients by different vaccination strategies. However T cell mobilization and its detection depends on the knowledge of tumor antigens and its epitopes. A number of tumor antigens have been identified but the knowledge of Th epitopes derived from these antigens is still limited. The first aim of the present study was to identify HLA-DRB1*0301-presented epitopes derived from Tyrosinase-related protein-2 (TRP-2), a member of the group of melanoma differentiation antigens. The search for TRP-2-derived Th epitopes was carried out by competitive *in vitro* peptide binding studies with predicted HLA-DRB1*0301 ligands and peptide/protein immunizations of HLA-DRB1*0301 transgenic mice. Via this strategy TRP-2_{60-74} was identified as a sequence containing a $CD4^+$ Th cell epitope. Importantly, the repeated TRP-2_{60-74} peptide stimulation of peripheral blood lymphocytes (PBL) from HLA-DRB1*03^+ melanoma patients led to the establishment of peptide-reactive $CD4^+$ Th cell lines which specifically recognized target cells loaded with recombinant TRP-2 protein. Even short term peptide stimulation of patients PBL revealed the presence of TRP-2_{60-74}-reactive T cells in some donors, suggesting *in vivo* activation of epitope-specific T cells.

Most tumor antigens, including TRP-2, are self-antigens, which have to be delivered in a more immunogenic format during vaccination, in order to break tolerance and to elicit strong immune responses in cancer patients. This may be achieved by delivery of tumor antigens/antigenic peptides by suitable bacterial vectors, like the facultative intracellular microbe *Listeria monocytogenes*. Thus, the second aim of the present work was to provide knowledge about adaptive immune responses against the bacteria in humans, in terms of $CD4^+$ T cell responses specific for the cytolysin Listeriolysin O (LLO), a major virulence factor of *Listeria* which has been identified as an immunodominant T cell antigen in infected mice. It was demonstrated that spleen cells from *Listeria*-infected HLA-DRB1*0301 transgenic mice specifically reacted against the epitopes $LLO_{202-213}$ and $LLO_{416-427}$, with $LLO_{416-427}$ being conserved in cytolysins from other bacteria. Specific $CD4^+$ memory T cell responses against $LLO_{202-213}$ and $LLO_{416-427}$ could be detected in normal HLA-DRB1*0301 individuals, suggesting that LLO is also an immunodominant target antigen in humans.

In summary, a novel epitope TRP-2_{60-74} presented by HLA-DRB1*0301 has been identified, which might be useful for vaccination and immunomonitoring of HLA-DRB1*0301 melanoma patients. Furthermore, two HLA-DRB1*0301-presented T cell epitopes from LLO have been characterized, which will be of relevance for clinical studies intending to employ attenuated *L. monocytogenes* strains as vectors for heterologous T cell vaccines in infectious and malignant diseases.