OUT OF FRAME BCR-ABL FUSION PEPTIDES AS TARGETS OF THE IMMUNOTHERAPY IN CHRONIC MYELOGENOUS LEUKEMIA

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Characterization of tumor associated antigens is an important step in the development of new immunotherapies of human cancers. Recently, new potential tumor specific antigens are described in BCR/ABL positive leukaemias: beside the main bcr/abl hybrid fusion transcripts, a small amount of different fusion transcripts derived from the alternative splicing between BCR exon 1, 13 and 14 with ABL exons 4 or 5 were identified in more than 80% of the cases. These variants are expressed in minor amounts in chronic myelogenous leukemia (CML) and acute lymphocytic leukaemia (ALL) patients and show a change in abl reading frame. This shift in the reading gives rise to the synthesis of novel hybrid fusion proteins contain an initial regular Bcr portion attached to a large out of frame (OOF) portion (112 aa) derived from abl gene. This OOF protein portion is expressed only in leukemic cells, it has not homology with other human proteins known, and it may represent an excellent target for cancer immunotherapy.
In this study three peptides, named peptide A, B and C, 39-aminoacid-long each corresponding to a third of the whole OOF aminoacidic sequence, were tested for their capacity to elicit specific immune responses in HLA A 2.1 transgenic mice. We found that the OOF peptides A and C can induce the generation of specific cytotoxic T lymphocytes (CTLs) able to kill "in vitro" the target cells in a class I HLA A2.1 restricted manner. In this test we used as target cells the human chronic myelogenous leukemia K562 cell line transfected with the HLA A2.1 gene in order to induce MHC class I molecules expression on the cell surface, these cells are positive for chromosome Philadelphia and also for the alternative hybrid transcript BCR/ABL. "In vitro" stimulation with the peptide A specifically induced IFNγ production by immune splenocytes. The simultaneous use of the three peptides in the vaccination strategy provided an inadequate immunization, indeed, the "in vitro" IFNγ secretion detected in response to peptide A, as well as $CD8^+$ mediated lyses, was lost in the vaccination with the three peptides together. However, when peptide A or peptide B were used alone, they were able to induce a specific humoral immune-response and the antibodies synthesized were able to bind peptides presented in association with HLA A2.1 molecules on the tumoral cell surface. These data as well as proving the OOF peptides ability to elicit a specific immune response they also provide evidence that leukemic cells, positive for the alternative hybrid BCR/ABL transcript, such as HLA^+ K562 cells, can process and present, in association with HLA A2.1 molecules, peptides from abl OOF protein portion on their surface and that antigen-specific T cells can target these peptides. On the basis of our results we think that immunization with peptide A sequence derived from OOF protein portion of abl gene appears to be an effective approach to generate multiple and potent immune responses against OOF protein portion of abl and may be a candidate CML specific tumor antigen for the complete eradication of the leukemic clone.