Abstract

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MHC Class-II Expression Targeting (CrossTAg) for the Generation of Tumor-Antigen-Specific $CD4^+$ T Lymphocytes

Ellinger C., Wehner C., Wilde S., Schendel D.J., Milošević S.

Institute of Molecular Immunology, Helmholtz Zentrum München

Although adoptive T cell therapy with $CD8^+$ cytotoxic T lymphocytes (CTL) is a promising cancer immunotherapy, high efficacy in a clinical setting has not been established. To increase efficacy, supportive transfer of $CD4^+$ helper T cells might offer a possibility to enhance $CD8^+$ CTL responses. $CD4^+$ T lymphocytes are known to provide pivotal help for $CD8^+$ CTL as well as to have a critical effect on the generation of long lasting $CD8^+$ memory T cells. Therefore, the rapid and efficient isolation and characterization of tumor antigen specific $CD4^+$ T lymphocytes is of great interest.

For this we refined a system to efficiently load MHC class II molecules using in vitro transcribed mRNA (ivtRNA), employing an antigen-coupled targeting signal (CrossTAg). In contrast to recombinant proteins, quality controlled ivtRNA can be rapidly produced and carries no immunogenic contaminants. We observed that antigen presenting cells (APC) transfected with CrossTAg-antigen ivtRNA were efficiently recognized by $CD8^+$ and $CD4^+$ T cells simultaneously. Consequently, we used tumor antigen-CrossTag ivtRNA-transfected APC to prime whole PBL of a healthy donor. Using fluorescence-activated cell sorting for CD40 ligand (CD154) expressing $CD4^+$ T lymphocytes after antigen-specific restimulation we succeeded in isolating multiple $CD4^+$ T cell clones directed against different Cancer/Testis-antigens presented on different MHC class II molecules. Clones generated with this method can easily be characterized for their respective T cell receptor and restriction element. Furthermore, they can be used to identify new epitopes recognized by $CD4^+$ T cells and contribute to future therapeutic approaches.

Thus, using MHC class II expression targeting and sorting for activation-induced CD154 expression we were able to establish a high-throughput method for the isolation of antigen-specific $CD4^+$ T lymphocytes.