Cellular & Molecular Immunology (2018) 15, 182–184

www.nature.com/cmi

LETTER TO THE EDITOR

Multi-epitope vaccines: a promising strategy against tumors and viral infections

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Cellular & Molecular Immunology (2018) **15,** 182–184; doi:10.1038/cmi.2017.92; published online 11 September 2017

Immune responses play a critical role in fighting tumors and viral infections. An antigenic epitope is a basic unit that elicits either a cellular or a humoral immune response. A multi-epitope vaccine composed of a series of or overlapping peptides is therefore an ideal approach for the prevention and treatment of tumors or viral infections.[1–5] Although some multi-epitope vaccines have entered phase I clinical trials, for example, EMD640744 in patients with advanced solid tumors,[6] designing efficacious multi-epitope vaccines remains a great challenge. An ideal multi-epitope vaccine should be designed to include epitopes that can elicit CTL, Th and B cells and induce effective responses against a targeted tumor or virus (Figure 1).

Compared to classical vaccines and single-epitope vaccines, multi-epitope vaccines have unique design concepts[4–11] with the following properties: (I) they consist of multiple MHC-restricted epitopes that can be recognized by TCRs of multiple clones from various T-cell subsets; (II) they consist of CTL, Th and B-cell epitopes that can induce strong cellular and humoral immune responses simultaneously; (III) they consist of multiple epitopes from different tumor or virus antigens that can expand the spectra of targeted tumors or viruses; (IV) they introduce some components with adjuvant capacity that can enhance the immunogenicity and long-lasting immune responses; and (V) they reduce unwanted components that can trigger either pathological immune responses or adverse effects. Well-designed multi-epitope vaccines with such advantages should become powerful prophylactic and therapeutic agents against tumors and viral infections.

Current problems in the field of multi-epitope vaccine design and development include the selection of appropriate candidate antigens and their immunodominant epitopes and the development of an effective delivery system. Development of a successful multi-epitope vaccine first depends on the selection of appropriate candidate antigens and their immunodominant epitopes. The prediction of appropriate antigenic epitopes of a target protein by immunoinformatic methods is extremely important for designing a multi-epitope vaccine.[12,13] In our laboratory, we always use immunoinformatic tools to predict and screen the immunogenic T- and B-cell epitopes of the target antigens, then design peptides rich in epitopes or overlapping epitopes.[7,8,11,14,15] For the prediction of B-cell epitopes, multiple alignments of the target antigen are initially carried out using software from the European Bioinformatics Institute website (http://www.ebi.ac.uk/Tools/clustalw2). Then, the structure, hydrophilicity and flexibility, and transmembrane domains of the target antigen are predicted and analyzed by methods including GOR,[16] Hoop and Woods[17] and artificial neural network (http://strucbio.biologie.uni-konstanz.de). Antigenic propensity value is further evaluated using the Kolaskar and Tongaonkar approach (http://bio.dfci.harvard.edu/Tools/antigenic.pl). Finally, the B-cell-dominant epitope is determined by comprehensive analysis and comparison, and T-cell epitopes including MHC-I restriction CTL and MHC-restriction Th epitopes are predicted using the SYFPEITHI network database (http://www.syfpeithi.de/Scripts/MHCServer.dll/EpitopePrediction.htm). All the above-mentioned programs are provided on the EXPASY server (http://www.expasy.org/tools). Recently, we have developed a multi-epitope vaccine, named EBV LMP2m, which encodes multiple CTL, Th and B epitopes from the *EBV LMP2* gene.[11] Expression of the recombinant multi-epitope protein from the constructed multi-epitope vaccine gene was optimized to maximize its production in *E. coli*. The specific CTL and Th cell reactions and B-cell activation (serum-specific IgG and mucosal IgA antibodies) were analyzed in BALB/c mice immunized with the multi-epitope vaccine. The multi-epitope-specific serum

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Received: 22 July 2017; Accepted: 1 August 2017

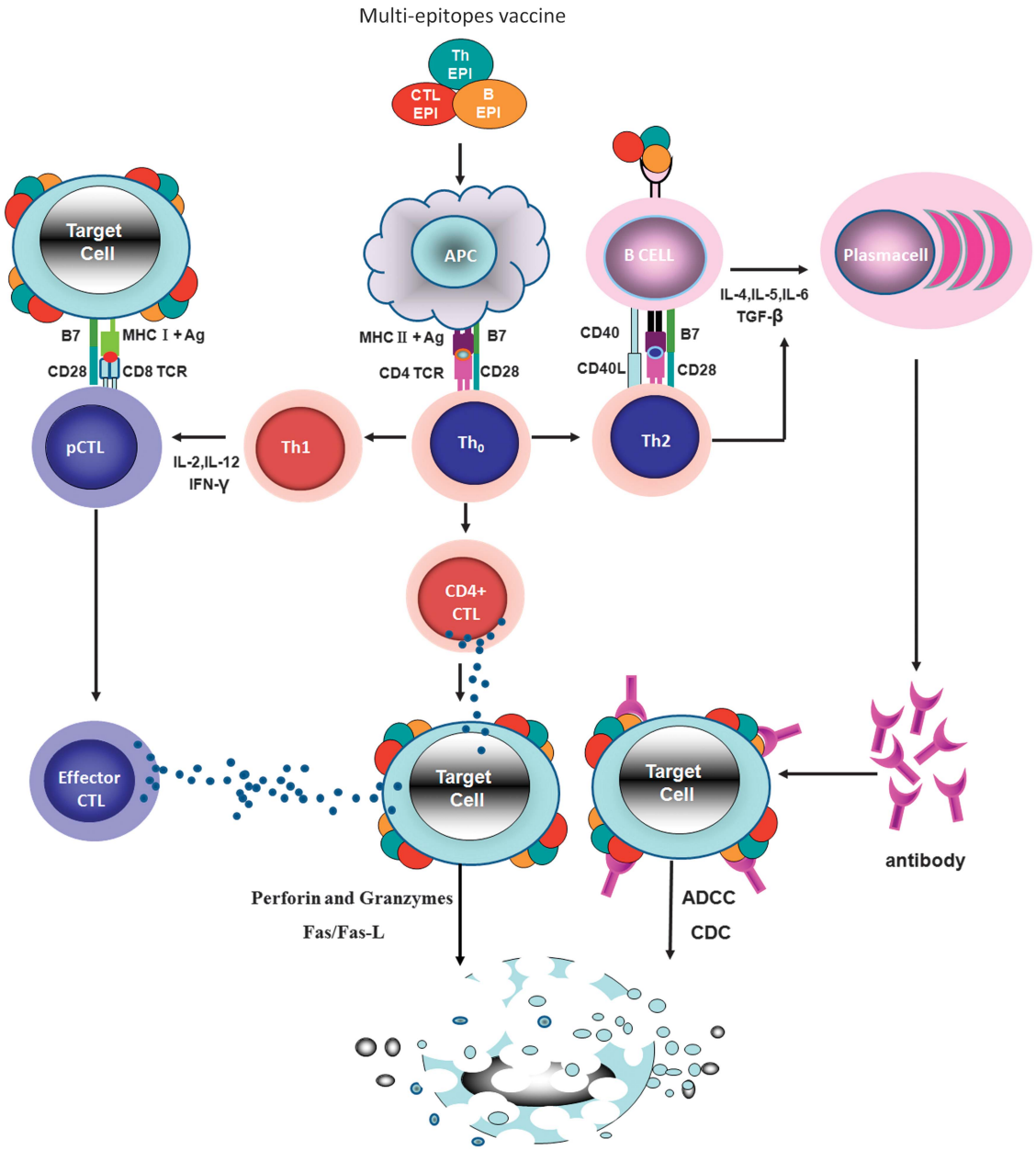

Figure 1 The molecular and cellular mechanism of immune responses induced by a multi-epitope vaccine. Multi-epitope vaccines can be composed of CTL, Th and B-cell epitopes in a series or overlapping epitope peptides. Through TCR, $CD8^+$ precursor CTL ($CD8^+$ pCTL) recognizes the complex of CTL antigen peptides bound to MHC class I molecules that are displayed by target cells (tumor cells or virus-infected cells). Antigen presenting cells (APC) take up the multi-epitope vaccine and present the Th antigen peptides bound to MHC class II molecules to Th0 cells. Th0 cells are differentiated into Th1, Th2 and $CD4^+$ CTL cells. Th1 cells secrete cytokines that stimulate $CD8^+$ pCTL to generate effector CTL cells, the latter of which will kill the target cells by both the perforin/granzyme and the Fas/FasL pathways. Th2 cells recognize the Th epitope bound to MHC class II molecules that are presented by B cells. After being activated, Th2 cells express CD40L molecules and secrete cytokines to stimulate B-cell activation; $CD4^+$ CTL cells secrete cytotoxins, directly killing the target cells by releasing granules containing perforin and granzyme B. B cells recognize and take up the B epitopes of the multi-epitope vaccine by BCR, presenting the Th epitopes bound to MHC class II molecules to activate Th2 cells. The B cells then proliferate and differentiate into plasma cells after binding to the CD40L molecules and cytokines provided by activated Th2 cells. The plasma cells secrete multi-epitope vaccine-specific antibodies to perform anti-tumor or anti-virus tasks in target cells by antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

antibodies in patients with EBV-related tumors (nasopharyngeal carcinoma) or EBV infection were analyzed using ELISA and western blotting. All the analyses demonstrated that the multi-epitope vaccine EBV LMP2m had a very high immunogenicity. Therefore, EBV LMP2m could be considered as a potential vaccine candidate and diagnostic agent.

The successful immunotherapy of a multi-epitope vaccine is also associated with an effective vaccine delivery system. At present, both virus-like particles (VLPs)[7,8,14] and nanoparticles[18,19] have been used as vehicles for delivering multi-epitope vaccines. We have used two types of VLPs: hepatitis B core antigen (HBcAg)-VLPs and hepatitis B surface antigen (HBsAg)-VLPs. Our data have shown that either HBcAg or HBsAg-VLPs multi-epitope vaccines strongly elicit specifically humoral and cellular immune responses and generate vigorous immune responses of the individual epitopes carried by the multi-epitope vaccine.[7,8,14]

In summary, multi-epitope vaccines can be considered as a promising strategy against tumors and viral infections. Immunoinformatics methods can help to predict and screen appropriate epitopes for designing an efficacious multi-epitope vaccine. Both VLPs and nanoparticles used for delivering a multi-epitope vaccine could increase its immunogenicity.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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