The Use of Filamentous Bacteriophage *fd* to Deliver MAGE-A10 or MAGE-A3 HLA-A2-Restricted Peptides and to Induce Strong Antitumor CTL Responses[1]

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Delivery of tumor-associated Ag-derived peptides in a high immunogenic form represents one of the key issues for effective peptide-based cancer vaccine development. We report herein the ability of nonpathogenic filamentous bacteriophage *fd* virions to deliver HLA-A2-restricted MAGE-A10$_{254-262}$- or MAGE-A3$_{271-279}$-derived peptides and to elicit potent specific CTL responses in vitro and in vivo. Interestingly, human anti-MAGE-A3$_{271-279}$-specific CTLs were able to kill human MAGE-A3$^+$ tumor cells, even if these cells naturally express a low amount of MAGE-A3$_{271-279}$ peptide-HLA epitope surface complexes and are usually not recognized by CTLs generated by conventional stimulation procedures. MAGE-A3$_{271-279}$-specific/CD8$^+$ CTL clones were isolated from in vitro cultures, and their high avidity for Ag recognition was assessed. Moreover, in vivo tumor protection assay showed that vaccination of humanized HHD (HLA-A2.1$^+$/H2-D^{b+}) transgenic mice with phage particles expressing MAGE-A3$_{271-279}$-derived peptides hampered tumor growth. Overall, these data indicate that engineered filamentous bacteriophage virions increase substantially the immunogenicity of delivered tumor-associated Ag-derived peptides, thus representing a novel powerful system for the development of effective peptide-based cancer vaccines. *The Journal of Immunology,* **2008, 180: 3719–3728.**

In the past decade, the identification and molecular characterization of many human tumor-associated Ags (TAAs)[4] recognized by CTLs has led to the development of new immunotherapeutic strategies of vaccination aimed at producing antitumoral CTL responses in cancer patients (1–4). Among TAAs, cancer/testis Ags, being expressed in many tumors of various histological types but not in normal tissues (with the exception of testis and placenta), are strictly tumor-specific and therefore ideal candidates for cancer vaccines (1, 4). Cancer/testis Ags include the MAGE family, constituted of several related Ags divided into three clusters, namely MAGE-A, MAGE-B, and MAGE-C, and they are expressed in a large variety of primary as well as metastatic tumors (2, 5, 6). In particular, MAGE-A3 and MAGE-A10 Ags have elicited considerable interest because they are expressed with high frequency in melanomas (~70% for MAGE-A3 and 50% for MAGE-A10) and in bladder, lung, esophagus, and head and neck carcinomas (~50% for MAGE-A3 and 35% for MAGE-A10) (2, 5–7). A variety of peptide epitopes present in the amino acid sequences of MAGE-A3 and MAGE-A10 Ags have been characterized (8, 9), and special interest has been bestowed upon HLA-A2 as a restriction element because of its frequency of occurrence in various ethnic groups (10). The nonapeptide-encompassing residues 271–279 and 254–262 from MAGE-A3 (MAGE-A3$_{271-279}$) and MAGE-A10 (MAGE-A10$_{254-262}$), respectively, are recognized by CTLs restricted by HLA-A2 (11, 12). Studies on T cell responses to MAGE-A3$_{271-279}$ and MAGE-A10$_{254-262}$ peptides, even in association with cytokines or presented by dendritic cells as APCs, have shown that specific CTL responses required repeated stimulations in vitro (11–16) and that repeated immunizations rarely generated CTL responses in vivo (17–21). Moreover, when the generation of peptide-specific CTLs could be achieved, CTLs might fail to recognize the peptide epitope on the neoplastic cell. This was the case of MAGE-A3$_{271-279}$ peptide-specific CTLs, which readily lysed HLA-A2$^+$/MAGE-A3$_{271-279}$ peptide-loaded target cells, whereas they did not recognize HLA-A2$^+$/naturally expressing MAGE-A3 tumor cells (13, 22). The lack of efficient tumor cell recognition by MAGE-A3$_{271-279}$ peptide-specific CTLs was due to the low abundance of peptide-HLA epitope complexes on the tumor cell surface, because of an impaired peptide processing in the neoplastic cell (22, 23). Taken together, these observations raise concerns on the immunogenicity of these MAGE peptide epitopes and, hence, on their usefulness as vaccines. Thus, the possibility to deliver TAA peptides in a high immunogenic form, capable of eliciting not only specific but also potent CTL responses able to recognize low amounts of Ag on the tumor cell, represents one of the key issues for the development of more effective peptide-based cancer vaccines.

Herein, we propose a novel Ag delivery system based on benign filamentous bacteriophage *fd* virions. We have described the ability of *fd* virions, engineered to display multiple copies of foreign

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[4] Abbreviations used in this paper: TAA, tumor-associated Ag; C_T, comparative cycle threshold; TCC, transitional-cell carcinoma.