THE CHARACTERIZATION OF SPONTANEOUS AND VACCINE-DRIVEN ANTIGEN-SPECIFIC CYTOTOXIC T LYMPHOCYTE RESPONSES IN MELANOMA PATIENTS

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OCTOBER 2004

TABLE OF CONTENTS

THESIS ABSTRACT

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Thesis submitted for the Degree of Doctor of Philosophy

The last decade has witnessed a huge expansion in the field of Tumour Immunology. A large number of tumour antigens recognized by T lymphocytes have been identified and new techniques have enabled the direct *ex vivo* analysis of epitope-specific cytotoxic T lymphocytes (CTL). These developments provide the tools with which spontaneous tumour antigen-specific CTL responses can be characterized in detail, have facilitated the development of antigen-specific tumour immunotherapeutic strategies, and have heralded a new era of immunomonitoring in clinical trials. In this work, *ex vivo* phenotypic and functional analyses of CTL specific for an HLA-A*0201-restricted epitope encoded by the melan-A tumour differentiation antigen (melan-A_{26-35}) were performed on samples from melanoma patients and normal healthy donors. *Ex vivo* tetramer analysis revealed circulating melan-A_{26-35}-specific T lymphocytes in 50% of both patients and normal donors. Phenotypic analysis of tetramer$^+$ cells was correlated with *ex vivo* assays of CTL function. In the normal donors and approximately 50% of patients, the melan-A_{26-35}-specific cells were always phenotypically and functionally naïve. However, in the remaining patients, a proportion of melan-A_{26-35}-specific cells were phenotypically antigen-experienced, and functional responses to peptide could readily be detected *ex vivo*. The observation in patients, that tumour antigen-specific CTL responses with effector function are "too little, too late", provided the basis for a clinical trial of recombinant plasmid DNA and Modified Vaccinia Ankara in patients with surgically-treated melanoma and a high risk of disease recurrence. Both vaccines were engineered to encode the same polyepitope string of seven HLA-A2- and HLA-A1-restricted tumour antigen epitopes, including the high affinity melan-A_{26-35} analogue. Immunomonitoring, which included detailed kinetic analyses of tetramer$^+$ cells, demonstrated that MVA was capable of generating an expansion of melan-A_{26-35}-specific CTL with effector function in approximately 50% of patients. However, no responses to the other tumour antigen epitopes were seen. A detailed analysis of CTL responses specific for recently-identified vaccinia-encoded epitopes (including a new epitope identified as part of this work) demonstrated that CTL responses specific for the viral vector were dominant over those for the recombinant epitopes. This finding has important implications for the future design of recombinant viral vaccines.