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Split T-cell tolerance as a guide for the development of tumor antigen-specific immunotherapy

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Tumor antigens NY-ESO-1 and p53 both frequently induce spontaneous serum antibody in cancer patients. While NY-ESO-1-specific $CD8^+$ and $CD4^+$ circulating T-cells occur mainly in NY-ESO-1-seropositive patients, p53-specific circulating $CD8^+$ and $CD4^+$ T-cells are respectively undetectable and common in most individuals. Understanding T-cell split tolerance can help define suitable targets for immunotherapy.

Since its discovery in 1997, we and other groups have investigated spontaneous and vaccine-induced immune responses against NY-ESO-1, a cancer/testis antigen expressed in many tumor types but not in somatic healthy tissues.[1] One of the key immunological characteristics of NY-ESO-1 is co-induction of spontaneous antibody, $CD4^+$, and $CD8^+$ T cell responses in cancer patients. Antigens that induce "integrated immune responses" are considered ideal targets for immunotherapy because adaptive immune subsets act in a collaborative manner for anti-tumor responses. Recently, the presence of integrated immune responses against NY-ESO-1 was shown to correlate with better clinical outcome after immunomodulatory treatment with CTLA-4 blockade.[2] Additionally, integrated immune responses against NY-ESO-1 in cancer patients can be induced or potentiated by proper vaccination. One limitation of the use of NY-ESO-1 in cancer immunotherapy is that its frequency can be low in individual tumor types and its expression pattern in tumor is often heterogeneous.[1] Thus, it is important to define other suitable antigens to expand the applicability of immunotherapy.

Another famous candidate for cancer immunotherapy is p53, a mutational tumor antigen. Recently, we reported spontaneous immune responses against p53 in comparison with those against NY-ESO-1 in ovarian cancer patients whose tumors frequently express NY-ESO-1 and/or accumulate p53 protein.[3] To enable a direct comparison of their immunogenicity, patients in the same study cohort were analyzed using the same experimental procedures for detection of spontaneous immune responses against p53 and NY-ESO-1. Circulating p53-specific serum antibodies were detected in about 20% of patients, a similar percentage to NY-ESO-1 serum antibodies found in this cohort. Remarkably, p53-specific $CD8^+$ T cell responses were not detected in p53-seropositive patients, nor in seronegative patients or healthy individuals, yet the same procedure detected clear NY-ESO-1-specific $CD8^+$ T cell responses in NY-ESO-1-seropositive patients in the same study cohort. These results suggest that the spontaneous activation and expansion of p53-specific $CD8^+$ T cells are strictly regulated, likely by peripheral/central tolerance due to ubiquitous expression of wild-type p53 both in peripheral and thymic antigen presenting cells.

On the other hand, p53-specific $CD4^+$ T cell responses were not only detected in 50% of patients who had p53 antibody, but also in the majority of seronegative cancer patients and healthy individuals, with similar magnitude and epitope distribution. Importantly, most p53-specific $CD4^+$ T cells in healthy donors were derived from $CD45RO^+$ memory T cell population, indicating that they were primed in vivo. This is in contrast to NY-ESO-1-specific $CD4^+$ T cells which are exclusively naïve in healthy donors and only readily detectable from the memory repertoire in NY-ESO-1-seropositive patients using our procedures.[4] It is unclear whether pre-activated p53-specific $CD4^+$ T cells seen in healthy individuals contribute to immunosurveillance, but they may help the strong and frequent induction of antibody responses once the tumor accumulates p53. These observations indicate that in contrast to $CD8^+$ T cells, $CD4^+$ T-cell tolerance to p53 is very weak or absent, as demonstrated in pioneering studies using wild-type and p53-deficient mice.[5,6] The difference in incidence of T-cell responses and tolerance profile between the two antigens may reflect ubiquitous expression of p53 in normal tissues vs. testis-restricted significant expression of NY-ESO-1 (**Fig. 1**). The former results in an ontogenic process of "split T-cell tolerance,"

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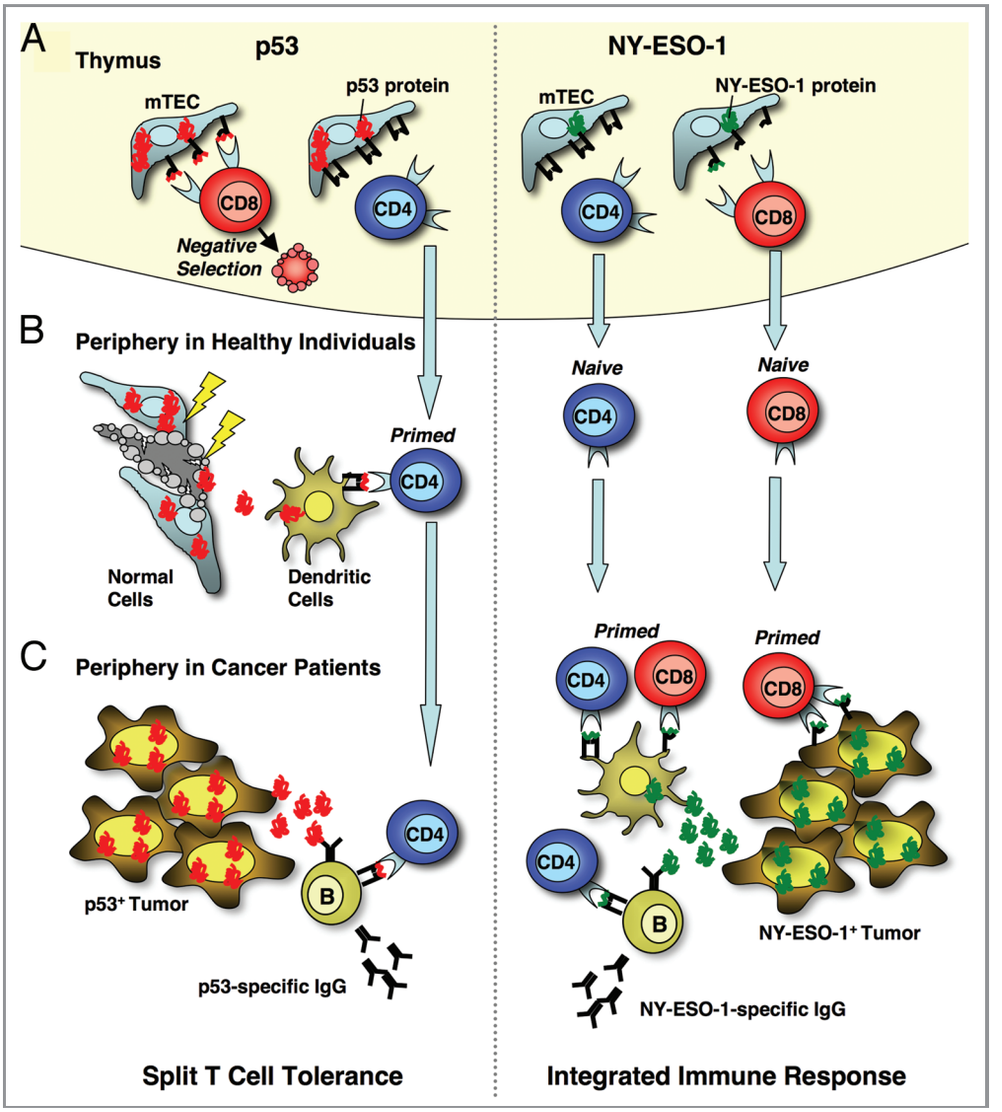

Figure 1. Model of spontaneous immune responses against p53 and NY-ESO-1. (A) In the thymus, medullary thymic epithelial cells (mTEC) constitutively expressing p53 eliminate high-avidity p53-specific $CD8^+$ T cells, while NY-ESO-1-specific $CD8^+$ T cells are capable of escaping negative selection. $CD4^+$ T cell central tolerance appears to have little effect for these antigens. (B) In the periphery of healthy individuals, normal cells upregulate p53 expression by cellular stress such as UV irradiation, NOS exposure, and malignant transformation, and release p53 protein by cell death. Dendritic cells capture p53 protein and activate $CD4^+$ T cells. In contrast, the testis-specific expression of NY-ESO-1 limits its spontaneous activation of specific T cells in healthy individuals. (C) In cancer patients, tumor cells expressing NY-ESO-1 and/or accumulating p53 protein release large amount of antigens that induce T cell activation and antibody production after uptake by dendritic cells and B cells, respectively.

limiting the usefulness of p53 for immunotherapeutic development.

Through immunomonitoring of other cancer antigens, we found that spontaneous $CD8^+$ T-cell responses against MAGE-A3 and NY-CO-58[7] are also limited when compared with NY-ESO-1. Indeed, NY-CO-58, an antigen with low-level expression in normal tissues but overexpressed in cancer cells, rarely elicits $CD8^+$ T-cell responses but is immunogenic for $CD4^+$ T cells in cancer patients and healthy donors alike. Even among cancer-testis antigens, MAGE-A3 elicits fewer $CD8^+$ T-cell responses spontaneously or after vaccination compared with NY-ESO-1. Why only some tumor antigens such as NY-ESO-1 are able to strongly induce functional $CD8^+$ T-cell responses is still to be determined. To explain differential induction of $CD8^+$ T-cell responses, yet similar $CD4^+$ T cell and/or antibody responses among tumor antigens, understanding $CD8^+$ T cell-specific regulatory mechanisms is required. Suppression in the periphery or poor antigen cross-presentation may lead to attenuated $CD8^+$ T cell responses, and involvement of central tolerance has been explored. Recently, it was demonstrated in a mouse model that expression of mouse cancer-testis antigen P1A in thymus limits the magnitude and avidity of P1A-specific $CD8^+$ T cells.[8] Although it was shown that medullary thymic epithelial cells express tissue-restricted cancer antigens including

NY-ESO-1,[9] the quantitative difference among different antigens is not known. Because expression of tissue-restricted antigens by AIRE appears to be probabilistic,[10] analyses of expression level at the single cell level may give further understanding of the central tolerance of tumor-specific $CD8^+$ T cells.

Analyzing spontaneous immune responses in cancer patients is an efficient strategy to learn about the immunogenicity and immune-regulating mechanisms of tumor antigens as a preclinical step for immunotherapy development. Antigen-specific immunotherapies should also incorporate strong immunomodulatory strategies such as anti-CTLA-4, anti-PD-1, or anti-GITR antibodies that further enhance the immunogenicity of tumor antigens. Our study comparing spontaneous immune responses against p53 and NY-ESO-1 revealed a critical difference in the regulation of $CD8^+$ T cell responses. Various antigens that are differently expressed in tumors and normal tissues have been identified and reported to be immunogenic using various immunomonitoring techniques that have different accuracy and sensitivity. To identify suitable targets for cancer immunotherapy, it is important to comparatively re-evaluate the immunogenicity of candidate antigens using standardized immunomonitoring techniques, to discover new targets eliciting integrated immune responses over split T-cell tolerance.

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