Th1 epitope selection for clinically effective cancer vaccines

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New cancer immunotherapies mark progress in our understanding of tumor biology and harnessing the immune system’s management of self. However, protein- and peptide-based vaccines are not yet consistently efficacious. Recent work uncovers principles governing the genesis of T helper type-restrictive immunity to self-antigens elicited by vaccine epitopes, enabling vaccines to skew the balance from tolerogenic Type II (Th2) to inflammatory Type I (Th1) T cells, and invigorating this cancer immunotherapeutic approach.

Many promising new agents under development for the immunotherapy of cancer block immunosuppressive checkpoints that, when commandeered by tumors, prevent the existing endogenous cancer-specific immune response from inhibiting tumor growth. The success of this strategy has at least 2 key prerequisites. The first is an underlying tumor-specific T-cell response that is vigorous enough to induce an antitumor effect when unleashed. The second is that those T cells be predominantly of a Type I helper T (Th1) phenotype, since a derepressed or augmented Type II (Th2) response will only serve to further antagonize the lytic function of cytotoxic effector T cells present in the tumor microenvironment. These caveats invoke the importance of installing the appropriate “gas pedal” to accompany the release of “brakes” in cancer immunotherapy. Type I helper T cells are found at very low levels within the tumor and in the peripheral blood of most cancer patients. As an example, in breast cancer, less than half of all patients have any evidence of CD8+ T cell infiltration into their tumors.1 In addition, when evaluating the peripheral blood of patients with breast cancer for T cells specific to common tumor antigens, one finds that interferon-gamma (IFNγ)-secreting CD4+ T cells and CD8+ T cells recognizing antigens such as carcinoembryonic antigen (CEA) and melanoma-associated antigen 3 (MAGE-A3) are significantly diminished compared to cytomegalovirus (CMV)-specific Type I T cells in the same patients.2 T cells generated by exposure to tumor antigens in vivo are most often Type II T cells. Factors produced by tumors can influence antigen presenting cells (APC) to secrete cytokines promoting the development of Th2 phenotypes.3 Cytokines secreted by Th2 cells, as well as other cells in the tumor environment, will suppress the development of Th1 and limit the generation of cytotoxic T cells.4 Further, tumor antigen-specific regulatory T (Treg) cells have been identified circulating in the peripheral blood of cancer patients enhanced by antigen-specific vaccination.5 Tregs further drive the development of Th2 cells by secreting interleukin-10 (IL-10) and transforming growth factor-β (TGFβ). The therapeutic value of tools for affecting the immune response, such as vaccines, that cultivate specific immune cell types has long been recognized.

Tumor-specific T-cell populations can be augmented through active immunization. Two of the most common methods of immunization are to vaccinate with whole proteins either purified or encoded in various constructs or with peptide sub-units of proteins designed to elicit either CD4+ or CD8+ T cells (Fig. 1A). For many vaccines, the adjuvant used in immunization is meant to provide the danger signal requisite to bias immunity to a Th1 phenotype.6 For self-antigens, such as those used to vaccinate against cancer, potent adjuvants may not be enough to inhibit Th2 formation. To address this point, a pre-clinical model of immunization against CEA encoded in a virus also carrying co-stimulatory molecules has provided proof-of-principal, demonstrating the generation of both antigen-specific Th1 and Th2 cell types.7 Conceptually, a method of immune intervention linked more closely to the initial immune response than exogenous addition of adjuvants might provide therapeutic advantages.

Recent studies have shown that self-tumor antigenic proteins contain both Th1- and Th2-inducing epitopes (Fig. 1Ai). Importantly, self-proteins can be screened for immunosuppressive epitopes, and removal of these segments of the protein has been shown to give rise to immunogenic peptides capable of selectively inducing Type I helper T cells (Fig. 1Aii).8 Web-based algorithms were used to identify putative major histocompatibility complex Class II (MHCII) epitopes of insulin-like growth factor binding protein-2 (IGFBP-2), a non-mutated self-tumor antigen, estimated to bind with higher affinity across a variety of human leukocyte antigen-DR (HLA-DR) alleles. Identified peptides were constructed and subsequently used to stimulate peripheral
blood mononuclear cells (PBMC) that were then screened for the presence of T cells secreting IFNγ or IL-10. Th cell phenotype screening across a population of patients selected peptides that elicited a predominant Th2 (IL-10) or Th1 (IFNγ) response, or induced secretion of both cytokines. Generation of T-cell lines with Th2 or Th1 selective peptides revealed that (1) peptide-specific T cells respond to intact protein presented by autologous APCs, thus, the peptides constitute native epitopes of IGFBP-2, and (2) the IGFBP-2-specific Th2 cells demonstrated a significantly higher functional avidity to stimulating peptides than antigen-specific Th1 cells. The observation that the IGFBP-2 epitope-specific Th2 cells require substantially lower amounts of antigen to be stimulated to proliferate compared to their Th1 counterparts suggests that Th2 lymphocytes would be the dominant cells elicited in vivo via antigen processing and presentation in the tumor environment (Fig. 1Bi). Thus, vaccines engineered to include only Th1-inducing epitopes could allow unfettered and selective generation of Th1 cells (Fig. 1Bii). High frequencies of antigen-specific Th1 immune cell trafficking to the tumor could work to reverse the established Th2-dominated environment, activating APCs and facilitating cross-priming (Fig. 1Bii).

IGFBP-2-specific vaccines were designed successfully to elicit either a Th2 (Fig. 1Ci) or Th1 (Fig. 1Cii) T-cell response. Infusion of IGFBP-2-specific Th1 cells in adoptive transfer mediated an antitumor response whereas infusion of the vaccine-induced Th2 cells had no effect on tumor growth. Vaccination with Th1-inducing epitopes also resulted in tumor inhibition, whereas the Th2 based epitope vaccine had no effect. Compellingly, when the Th1 and Th2 vaccines were equally admixed, the inclusion of Th2 epitopes negated the
antitumor effect of the Th1-inducing vaccine. Presumably the Type II cytokine secretion by the Th2 cells limited Type I T-cell function (Fig. 1Di). Th1 selected epitopes drive a robust antitumor response to vaccination (Fig. 1Dii). The clear functional difference between these epitope sequences begs the question of what differentiates them from one another fundamentally, in other words, what features determine a Th1- versus a Th2-stimulating sequence?

The amino acid sequence of peptides presented in MHC complex has long been known to be the first signal driving differentiation of immature CD4⁺ T cells into cell-fate restricted Th1 or Th2 cells. Th2-inducing peptides demonstrate numerous homologies to bacterial antigens, and other self-antigens, whereas Th1-biasing peptides displayed limited homology with other proteins. Prior investigations have demonstrated that T cells specific for IGFBP-2 peptides are cross reactive against highly homologous pathogen-derived proteins as well as IGFBP-2. Furthermore, the incidence and magnitude of Th2-based immune responses against IGFBP-2 have been found to exceed that of Th1-immunity in individuals screened for Type I and Type II responses. Ongoing studies are evaluating the relationship between the IGFBP-2 Th2-inducing peptides with homologous bacterial proteins that are commonly expressed by flora potentially present in the gastrointestinal tract. The higher magnitude and incidence of these cells may relate to a homeostatic process associated with endogenous microbiota.

With the recent successes of checkpoint inhibitors for cancer therapy against some malignancies, the need for effective vaccines that efficaciously induce Type I cancer-specific immunity is pressing. The majority of patients may require T-cell immunity to be elicited before it can be “unleashed.” Many patients will benefit from increasing the frequency and activity of Th1 cells in the existing Th2 tumor environment prior to other immune therapies. Selective Th1 vaccines could become a critical component comprising future cancer immunotherapeutic regimens.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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