Chapter

Mimetic Vaccines in Immuno-Oncology

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Abstract

While the interest in cancer vaccines is renewed by some results in vaccine-based clinical trials, the premise still suffers from the incomplete concept of a successful vaccine. Future progress may come from matching preclinical data with clinical expectations while taking a step back to understand the systems perspective. A field that benefits most from this bird's eye view is tumor immunology. For instance, the accumulation over the last three decades of clear associations of T and B cell cross-reactivity between a set of host targets of autoimmunity and microbial antigens strongly supports a pathogenic role for molecular mimicry. Mimicry on its turn invites the concept of networks of molecular interactions. The intentional and rational approach to exploit mimicry in cancer vaccine development, while littered with failure, has provided also some insight into success. Here, we visit successes and underlying rationale to lend to future development of mimetic vaccines in immune-oncology.

Keywords: vaccine, anti-idiotype, peptide, tumor associated carbohydrate antigens, carbohydrate mimetic peptide

1. Introduction

Targeting malignancies through manipulating the immune system has seen success in a variety approaches ranging from whole cell vaccination, to autologous dendritic cell based vaccines and therapeutic immune-modulation [1–5]. But a number of opportunities and challenges remain. While tumor antigen identification from sequencing the cancer genome continues to be a high priority we now know that tumor antigens arise from multiple mechanisms that include somatic mutations, translocations, and amplifications and post-translational modifications. The role of post-translational modification with tumor associated carbohydrate antigens (TACA) in the generation of novel cancer antigens is in particular an opportunity to be explored [6–9].

Characterizing and overcoming the immunosuppressive environment of the tumors has led to a focus on downstream checkpoints that regulate activated T cells, or on vaccination and T cell adoptive transfer to expand the T cell pool [10–12]. However, it is well known that cancer-signaling pathways play pivotal roles in the biologic behavior of tumor cells that creates an opportunity to rethink cancer in general [13] and rethink cancer targeting strategies with small molecules [14, 15], with monoclonal antibodies [16] and induced antibodies [17, 18]. By the same token such pathways are also involved in developing therapeutic resistance, which

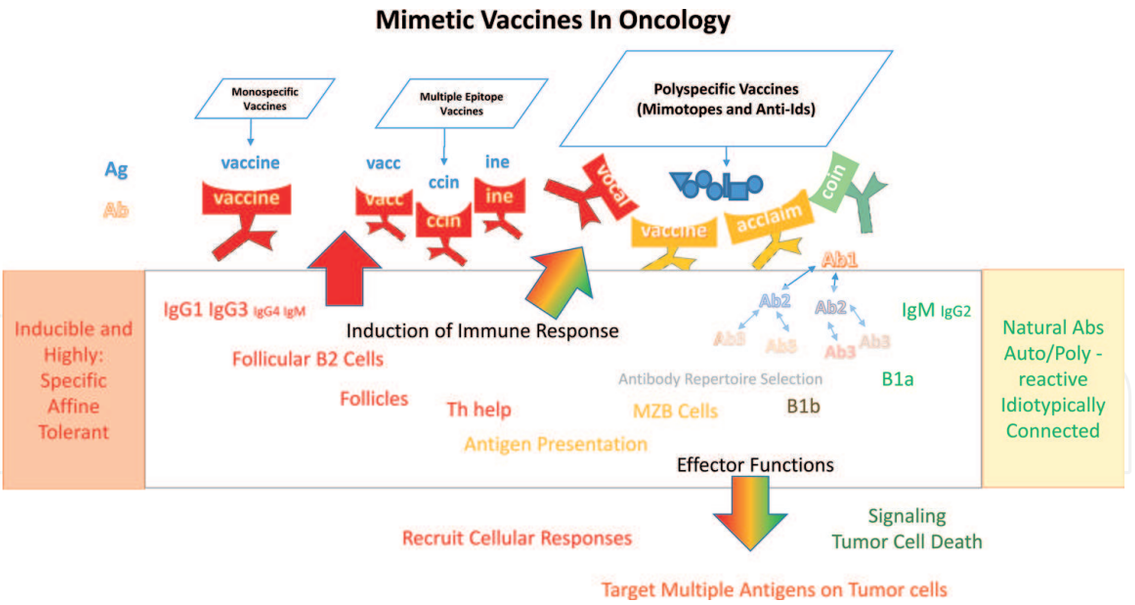

Figure 1.
The concept of mimetic vaccines in oncology. On the one end the spectrum of B cell subsets includes high affinity/specificity clones generated by somatic hypermutation in B2 follicular cells under conditions of strict tolerance to self. On the other, are the innate like B1a cells producing constitutively poly/autospecific natural antibodies. Carbohydrate specificity and anti-idiotype interactions are related more to the later compartment. Polyspecific vaccines based on carbohydrate mimotopes or idiotypes recruit B cell clones across that spectrum but their novel properties are related mostly to their capacity to elicit diversified responses from MZ and B1 cells including idiotypically connected clones. In addition, the mimotopes capture only the most salient features of the carbohydrate epitopes and induce diversified responses targeting multiple antigens (illustrated by diverse words sharing only partially the topology of the mimotope as compared to highly specific responses that match the shape of the epitope). Thus, mimetic vaccines both target polyspecific compartments of the B cell repertoire as well as they themselves function as polyspecific antigens.

requires alternative immunotherapeutic strategies. One such strategy is to develop polyclonal humoral immune responses by active immunotherapy. Itself, this concept can have multiple approaches and an orchestra of potential mechanisms that encompass a dynamic systems immunology perspective (**Figure 1**). On the one hand the effect can be pursued by formulating a platform with multiple epitopes of target antigens [19]. On the other—making use of polyspecific (pan-antigen) mimetics to target simultaneously multiple antigens on cancer cells [17, 18]. A polyclonal antibody approach would target more than two antigens on a single tumor cell, which is expected to have even higher potential. This latter idea is a part of the conceptual evolution in immune-oncology harnessing polyclonal responses to cancer cells.

2. Setting the stage: systems concepts

Systems immunology is now in focus to understand the immune system [20], especially in the context of vaccinology [21]. This perspective is ushering in a new era in vaccine development [22]. For the future, it is argued that successful approaches will depend on the elucidation of the entire network of immune signaling pathways that regulate immune responses with an eye toward integrating advances in computational and systems biology, genomics, immune monitoring, bioinformatics and machine learning [22, 23].

Systems immunology also teaches us that one antigen can substitute for another having the potential to regulate tolerance [24–28]. However, it is unclear why an immune system that is tolerant of its own self-antigens would respond to a self-antigen mimic in a vaccine. Antibodies referred to as anti-idiotypic are produced during

the process of tolerization and demonstrated in tolerant animals [29, 30] and in patients [31]. These antibodies may prevent a B cell receptor from interacting with the antigen. Jerne envisioned the immune system as a web of immunoglobulin V domains constituting an idiotypic network. Inherent to the idiotype network is that antibodies recognize antibodies. Jerne thought that regulatory processes governed by idiotypic interactions could explain the generation of the various immune states that include tolerance.

An extension of the network theory was that antibodies, by virtue of being recognized by antibodies, might function as mimics of antigens that would break tolerance instead of maintaining it—the so-called Ab2, used as antigen surrogates [32]. Thus a new context of molecular mimicry was born—one highlighted by the functionality of idiotypic antibodies in the context of the idiotype network theory [33–39].

Smaller fragments (peptides) of anti-idiotypes proved to translate successfully to vaccines too [40]. Peptides as mimics of antigens were clearly defined with the advent of phage screening technology [41, 42] growing in its application in biomedical sciences [43]. Peptide mimics are well defined as B and T cell epitopes [44]. Now there is an unprecedented opportunity to unravel the intricacies of the human immune response to immunization. Yet, fundamentally, vaccine strategies across susceptible disease depend on the identification of immunogenic antigens that can serve as the best targets [45–47].

Tumor antigens present a special challenge. Except for small details defined by mutations or altered post-translational modifications, generally they are self-antigens and this poses a barrier to effective vaccination. Tolerance is different from non-specific immunosuppression, and immunodeficiency. Like immune response, tolerance is specific existing both for T-cell and B cells and, like immunological memory tolerance is lasting longer at the T cell level than at the B cell level. Maintenance of immunological tolerance requires persistence of antigen. Tolerance can be broken naturally or artificially [48, 49]. Mimicry might impact on an already existing autoimmune process rather than precipitate novel disease by breaking of tolerance from the beginning [50]. While molecular mimicry is proposed as a basis for potential pathogenesis of some human disease, there are examples also of its exploitation in vaccine development.

3. Polyclonal activation

Now it is acknowledged that the natural antibody repertoire is created in the absence of exogenous antigens and/or germinal center maturation [51, 52]. It is also acknowledged that these preexisting antibodies can be affected by the presence of exogenous antigen since they recognize in a polyspecific manner evolutionarily fixed epitopes present in foreign antigens as well as on self-antigens [53]. Because of their constitutive expression, responses by natural antibodies are generally excluded from vaccine strategies. Among approaches that can modulate the natural antibody repertoire are immunizations affecting idiotypic interactions. When possible, an "idiotypic vaccination" could be a little explored way to activate the B and T cell cascades involving the natural responses against antigens.

Once acclaimed, idiotypy—the theory that the B lymphocyte repertoire forms a highly connected network of mutually recognizing and stimulating clones [54, 55]—unfortunately predated the discovery of many more levels of immune system complexity. The daunting task of attuning to the new knowledge prevented this theory from maintaining a support that would match its intellectual attractiveness. The first significant update, which almost rehabilitated it, stated that only the compartment of the B cell repertoire characterized by germline variable regions and

the prerequisite physiological poly/autoreactivity forms this network [56]. Almost, because many immune system phenomena like a self-assertive rather than ignorant tolerance or the immune memory ultimately do not need to be explained by emergent properties of the immune network. Now it is accepted that specific cell populations and genetic programs rather than the dynamics of a network of functionally equivalent agents (clones) are responsible for almost all of the observed immune phenomena. In fact, recent development in our understanding of swarms of simple agents uncovers the limits of such systems where complexity of the behavior of the agents and the size of the system have Goldilocks conditions for optimal behavior [57]. No wonder, evolution has used the "swarm" solution rarely and ultimately replaced it (complemented it) in most cases by centralized systems and specialized components at a higher level of organization.

Another reason the intellectually attractive "second generation network" hypothesis also fails is may be the fact that the compartment producing natural antibodies is defined in many ways and even 25 years later is still rather a fuzzy set [53]. While the natural antibodies in a strict sense are produced by a particular subset of B1 cell derived plasma cells in the bone marrow without external stimulus there are B1 cells (e.g. B1b) and marginal zone cells that produce antibodies with many "natural" characteristics like polyspecificity in response to stimulation [58]. Thus, focusing on the naturally autoreactive compartment of the repertoire did not answer all questions but also added another dimension of uncertainty.

Natural antibodies are known to bind to a variety of antigens that are both self and exogenous and thereby providing one of the first lines of defense against both bacterial and viral pathogens [53, 59]. Antibodies reactive to self-antigens play a key role in both healthy individuals and patients with autoimmune disorders [60–62]. Hence, such antibodies are intrinsically multifaceted in their regulatory roles in immune responses and tolerance. While the immune response activated against self can be detrimental when triggered in an autoimmune genetic background, tuning immune activity with natural antibodies is a potential therapeutic strategy. One conceptual approach in this tuning is using naturally occurring anti-idiotype (anti-Id) antibodies to stimulate multifaceted natural antibodies.

4. Anti-idiotypic antibodies as mimics

Anti-idiotypic based vaccines have a long history of generating immune responses in experimental animals and in humans [27, 28, 63–65]. One of the first demonstrations for the basis of molecular mimicry observed between proteins and anti-idiotypes for proteins was dissected in the TEPC-15 idiotype system [66]. Vasta et al. [66] illustrated that mimicry could be at the sequence level. They suggested that the minimal stretch of homology (8–10 amino acid residues) was responsible for the cross-reactive nature of the TEPC-15 idiotype and the acute-phase protein C-reactive protein (CRP) from the horseshoe crab *Limulus polyphemus* (limulin). Of no less importance, it was shown that T helper cells could recognize a shared determinant that is present on idiotypically different myeloma proteins [67]. These findings collectively showed that T helper cells, induced by priming with antigen, can recognize shared idiotypic determinates, suggesting that peptides derived from anti-idiotypes can be processed as immunogens [40, 68].

The early studies of anti-idiotypes made clear the idea that functional mimicry of ligands of biological receptors is a matter of just binding to an antibody-binding site. This functional or antigenic mimicry ushered in concepts and a technology. It was evident that structural and immunological rules governing molecular mimicry

require definition for its successful exploitation whether anti-idiotypes, small fragments derived from them or peptide mimetics [69, 70]. It was suggested that ligand-based pharmacophore design principles could be applied to designing peptides that can mimic ligands reactive with antibodies [69, 71]. Often times it was stated that there were no observable structural correlations to explain the mimicry [72]. Yet it seemed that antibodies could mimic antigens at the molecular level whereby the antigen and anti-idiotype could bind essentially the same combining-site residues of the Ab1 antibody [73].

Historically, clinical trials with anti-idiotypes in the cancer space have proved to be of mixed success [74, 75] but, clearly showing that humoral and cellular immune reactivity against a tumor can be enhanced upon active anti-id vaccination [76]. Other studies with anti-ids in humans have included those associated with tumor associated carbohydrate antigens (TACAs), [77–80]. An anti-Id vaccine, Racotumomab, raised against the murine anti-ganglioside N-glycolyl (NGc) GM3 (NGcGM3) has shown efficacy [81] in several phase I trials in melanoma, breast and lung cancers [82, 83]. These examples are representative for other anti-Id vaccine trials. In sum they indicate the induction of B and T-cell immune responses against a tumor.

5. Mimetic peptides in immuno-oncology

From a technology perspective the concept of developing and screening combinatorial or random peptide phage display became an effective means of identifying peptides that can bind target molecules and regulate their function [41, 42]. Phage-displayed peptide libraries have proved effective for (i) mapping of B and T cells epitopes, (ii) defining bioactive peptides that bind to receptors, (iii) selection of cell/organ specific binding peptides, and (iv) identification and development of peptide-mediated drug delivery systems to mention a few applications [43]. Among concepts emphasized by phage screening technology was that of the mimotope. The term mimotope, coined by Mario Geysen in 1986 [84] described a peptide mimicking a discontinuous antigenic determinant on foot and mouth virus. Phage screening technology has evolved, giving us unparalleled access to tight binding peptides to significantly accelerate identification of new leads for drug discovery [85].

The ability to produce combinatorial peptide libraries with a highly diverse pool of randomized ligands has transformed phage display into a straightforward, versatile and high throughput screening methodology for the identification of potential vaccine candidates against different diseases that include cancer [86–88]. While most studies with mimotopes identified by phage screening are still in preclinical studies, immunization results do provide insight for future development of novel mimotope-based tumor vaccines [89–92]. Starting from phage screening, we have developed carbohydrate-mimetic peptide (CMP) vaccines that target carbohydrate antigens [70, 71]. We brought CMPs from preclinical assessments of mimicking peptides of TACA [93–95] to clinical studies [17, 18] where one peptide can induce polyclonal responses to two or more antigens, which do or do not share epitopes.

Clinically we have shown that CMPs can achieve this multi-epitope targeting. The peptide P10s is a CMP designed to mimic both LeY and GD2 antigens using anti-LeY (BR55-2) and anti-GD2 (ME36.1) antibodies as templates [95]. Therefore, vaccination with P10s may lead to targeting various molecular entities associated with glycoproteins and with glycolipids, reducing the possibility of immune editing and escape. Moreover, the P10s vaccine has the potential to activate cellular responses [96]. We completed a phase I clinical trial of the P10s

vaccine in breast cancer patients and showed its feasibility, safety and immune efficacy. The data indicates induction of anti-peptide and anti-glycan antibodies [17, 18]. Antibodies of immunized subjects mediated cytotoxicity on human breast cancer cell lines through currently unknown mechanisms independent of complement-mediated cell cytotoxicity, but had no effect on normal breast cell line MCF-10A [17, 18]. Serum antibodies parallel the effect of anti-LeY and anti-GD2 monoclonal antibodies. After more than 6 years of follow up, 4 out of 6 vaccinated subjects are still alive with 3 of them in remission. Our clinical data suggest that vaccination of breast cancer patient's results in tumor regression and survival benefit.

6. Linking signaling with polyclonal response

The complexity of glycans found on the cell surface argues for their informational role involved in regulating multiple cellular processes essential for tumor development or its metastases. Since TACAs are expressed on glycoproteins and glycolipids that regulate multiple cellular pathways, TACAs are by definition pan-targets. Many glycoproteins and glycolipids are associated with signaling cascades through Focal Adhesion Kinase (FAK) with its activation hypothesized to play an important role in the pathogenesis of human cancers [97, 98]. FAK is a non-receptor tyrosine kinase that plays an important role in signal transduction pathways that are initiated at sites of integrin-mediated cell adhesion and by growth factor receptors [99]. FAK is also linked to oncogenes at both a biochemical and functional level. Moreover, overexpression and/or increased activity of FAK are common in a wide variety of human cancers, implicating a role for FAK in carcinogenesis. It is therefore a key regulator of survival, proliferation, migration and invasion: signaling cascades and processes that are all involved in the development and progression of cancer. FAK localized at focal contact sites and communicates with TACA-expressing molecules. Coordinated and localized stimulation of these cascades influences focal contact turnover and actin cytoskeleton dynamic addition to expression of motility- and invasion-associated proteins such as matrix metalloproteinases. FAK-dependent regulation of chemokine's and cytokines in cancer cells can drive elevated levels of regulatory T cells into the tumor environment resulting in suppression of the anti-tumor CD8+ T-cell response [100].

FAK is associated with several mechanisms to regulate cell migration and invasion through its phosphorylation. These include interactions with Src, P13K, Grb7, N-WASP and EndoII. Interaction with integrin also mediates FAK association with extracellular matrix, triggering the binding of adaptor molecules leading to the modulation of small GTPases, Ack, ERK2/MAP and JNK/SAP kinase cascades. The convergence of signaling by FAK plays an important role in tumor-cell survival and in drug resistance, as these pathways overlap. Given the important role of FAK in a large number of processes involved in tumorigenesis, metastasis, and survival signaling, Akt/FAK pathways are now regarded as a potential target to overcome drug resistance.

A variety of results suggest that GD2 and LeY play a role in the migration and survival of cancer cells, since (i) anti-GD2 antibodies [101] and natural anti-TACA antibodies [102] can mediate anoikis; (ii) apoptosis signals are transduced via reduction in the phosphorylation levels of FAK, the activation of a MAPK family members, p38 and c-Jun terminal kinase (JNK), upon binding of such antibodies [101, 103]; (iii) P10s reacts with anti-GD2 and anti-LeY monoclonal antibodies; (iv) anti-P10s antibodies block cell migration and (v) anti-P10s antibodies from P10s immunized subjects are cytotoxic to human breast cancer cell lines.

7. Synergism of chemo- and immunotherapy

Combining agents with distinct or perhaps overlapping mechanisms of action can potentially result in synergistic anticancer effects. Numerous preclinical studies have established the synergistic relationships between modulation of Tregs and differential expression of immune effector ligands on tumor cells [104, 105]. Consequently, combinatorial anticancer therapy is now a well-established paradigm due to a number of clinical trials demonstrating therapeutic success. However, the mechanisms associated with successful application are not well understood [106]. Standard cancer chemotherapy can promote tumor immunity in two major ways: (i) inducing immunogenic cell death as part of its intended therapeutic effect leading to epitope spreading [107, 108]; and (ii) disrupting strategies that tumors use to evade the immune response [109, 110]. In particular, epitope spreading ensures a polyclonal, polyfunctional immune response that promises to keep the tumor in check indefinitely [111]. Cancer patients can display tumor-reactive antibodies at baseline, which can increase in both breadth and quantity after immunotherapy [112]. IgG antibodies, produced by B cells, are indicative of CD4 helper T cells of linked specificity. Activation of tumor-specific CD8 T cells result from the same processes that generate activated CD4 T cells.

Checkpoint inhibitors have changed the face of immunotherapy with objective responses observed in some patients based on combinatorial regimes involving CTLA4 agent ipilimumab and the PD-1-specific checkpoint inhibitor nivolumab [113]. Nevertheless, a sizeable fraction of patients do not respond to checkpoint inhibitor combination. This could be for several reasons that include but are not limited to not having the correct T cell precursors to target the tumor associated antigens, dysfunctional T cell receptors and down regulation of MHC complexes [114, 115]. Interestingly, FAK inhibition has been noted to increase the activity of checkpoint inhibitors [116]. This work suggests that FAK inhibition increases immune surveillance and renders tumors responsive to immunotherapy.

In our own work when combining chemotherapy with CMPs we primarily focused on immune effector synergistic relationships. A particular synergistic action requires apoptotic tumor cell death, and does not occur as a consequence of perturbations in immunological regulatory circuits. The resistance of many types of cancer to conventional chemotherapies is problematic and a major factor undermining successful cancer treatment. Again FAK plays a role with its silencing augments docetaxel-mediated apoptosis of cancer cells [117]. We have shown that immunization with P10s can overcome resistance to taxanes and coupled with other studies indicating that targeting GD2 antigen is associated with FAK silencing we have come to another important therapeutic option of inducing multiple responses that go through the FAK gatekeeper to improve upon immunotherapy strategies.

8. Conclusion

Harnessing the body's own immune system to kill cancer cells has shown promise for a growing number of cancers, revolutionizing the clinical management of multiple tumors. The success of checkpoint antagonists heralds the dawn of a new age in cancer therapy, in which immunotherapy is becoming a key strategy for clinical management. Checkpoint inhibitors have taught us that they can unleash natural responses to tumor cells. The goal of cancer vaccines should be rethought in terms of boosting those natural responses. Combination therapies that integrate distinct therapeutic modalities that include vaccines, small molecules, radiotherapy and checkpoint inhibitors are under investigation. Yet understanding the cellular and

molecular underpins are essential for effective translation to the clinic. Polyclonal activation of the immune system should lend to epitope spreading phenomena, which will further effectiveness of cancer therapy. Yet this concept had been for the most part limited to the idea of presenting multiple epitopes of a particular target associated with T cell activation. This viewpoint needs to be reassessed to include the idea of extending to humoral responses since antibodies have proved to be essential to the cancer treating armament.

Conflict of interest

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. TKE and AP are named as inventors on an institutional patent application filled by UAMS that is related to the CMP vaccine briefly described in this manuscript. Therefore, TKE and AP and UAMS have a potential financial interest in the vaccine briefly described. No financial or other support of any kind has resulted from this patent application. These financial interests have been reviewed by approved supervision in accordance with the UAMS conflict of interest policies.

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