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REVIEW

K. L. Knutson · M. L. Disis

Tumor antigen-specific T helper cells in cancer immunity and immunotherapy

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Abstract Historically, cancer-directed immune-based therapies have focused on eliciting a cytotoxic T cell (CTL) response, primarily due to the fact that CTL can directly kill tumors. In addition, many putative tumor antigens are intracellular proteins, and CTL respond to peptides presented in the context of MHC class I which are most often derived from intracellular proteins. Recently, increasing importance is being given to the stimulation of a CD4+ T helper cell (Th) response in cancer immunotherapy. Th cells are central to the development of an immune response by activating antigen-specific effector cells and recruiting cells of the innate immune system such as macrophages and mast cells. Two predominant Th cell subtypes exist, Th1 and Th2. Th1 cells, characterized by secretion of IFN-γ and TNF-α, are primarily responsible for activating and regulating the development and persistence of CTL. In addition, Th1 cells activate antigen-presenting cells (APC) and induce limited production of the type of antibodies that can enhance the uptake of infected cells or tumor cells into APC. Th2 cells favor a predominantly humoral response. Particularly important during Th differentiation is the cytokine environment at the site of antigen deposition or in the local lymph node. Th1 commitment relies on the local production of IL-12, and Th2 development is promoted by IL-4 in the absence of IL-12. Specifically modulating the Th1 cell response against a tumor antigen may lead to effective immune-based therapies. Th1 cells are already widely implicated in the tissue-specific destruction that occurs during the pathogenesis of autoimmune diseases, such as diabetes mellitus and multiple sclerosis. Th1 cells directly kill tumor cells via release of cytokines that activate death receptors on the tumor cell surface. We now know that cross-priming of the tumor-specific response by potent APC is a major mechanism of the developing endogenous immune response; therefore, even intracellular proteins can be presented in the context of MHC class II. Indeed, recent studies demonstrate the importance of cross-priming in eliciting CTL. Many vaccine strategies aim to stimulate the Th response specific for a tumor antigen. Early clinical trials have shown that focus on the Th effector arm of the immune system can result in significant levels of both antigen-specific Th cells and CTL, the generation of long lasting immunity, and a Th1 phenotype resulting in the development of epitope spreading.

Keywords Vaccines · CD4 T cell · Helper T cell · Epitope · Tumor antigen

Abbreviations

ANN	Artificial neural networks
APC	Antigen-presenting cells
CTL	Cytotoxic T cells
DC	Dendritic cell
HLA	Human leukocyte antigen
IFN	Interferon
IL	Interleukin
TCR	T cell receptor
Th	T helper
Th1	T helper 1
Th2	T helper 2
TNF	Tumor necrosis factor

K. L. Knutson (✉)
Department of Immunology,
Mayo Clinic College of Medicine,
342C Guggenheim Bldg., 200 First St.
SW, Rochester, MN 55906, USA
E-mail: knutson.keith@mayo.edu
Tel.: +1-507-2840545
Fax: +1-507-2660981

M. L. Disis
Tumor Vaccine Group, Oncology, University of Washington,
1959 NE Pacific Street, Box 356527, Seattle, WA 98195-6527, USA

The pivotal role of T cell help in tumor immunity

T helper cells are central to the development of immune responses, for protection against infection and possibly

malignancy, by activating antigen-specific effector cells and recruiting cells of the innate immune system such as macrophages, eosinophils and mast cells [1–7]. Tumor antigen-specific Th cells can be activated by either antigen-presenting cells or directly by MHC class II-expressing tumors. The activation of the Th cell likely occurs when tumor antigens reach lymph nodes either through direct trafficking of tumor cells or antigen-presenting cells that have encountered antigen in the tumor bed. During early immune activation, APC delivery of antigens to lymph node T cells is probably the predominant step since tumor cell migration (i.e. metastasis) is limited in the early stage of disease. Indeed, cancers can be infiltrated with dendritic cells (DC) early in the course of a tumor. For example, approximately 30% of node-negative breast cancers have significant DC infiltration [8]. In addition to DC-mediated activation of tumor cells, there is some evidence that tumor cells can act directly as APC for activating Th cells. Of course, this would be dependent on tumor expression of MHC class II. It is generally believed that most tumor cells do not express MHC class II but in most tumor types a significant proportion of tumor cells express one or more MHC class II genes. Melanomas typically have a high expression of MHC class II compared to other tumor types. Tumors that can be associated with significant expression of MHC class II are melanoma, lung cancer, breast, and osteosarcomas [9]. It is estimated that more than 40% of melanoma tumor cells express one or more MHC class II molecules. Expression in lung cancers is dependent on the subtype. While squamous cell and small cell lung cancer consistently have low level expression, approximately 60% of adenocarcinomas and large cell carcinomas express some level of the molecules [10]. The study of MHC class II molecules in breast cancer has produced discordant results ranging from 25% expression to nearly 62% [11–13]. About one-third of osteosarcomas are positive for MHC Class II [14]. MHC class II-negative tumor cells can be induced to express MHC class II molecule oftentimes unless there is some underlying defect in IFN responsiveness [15, 16]. Antigen-primed Th cells can directly activate tumor antigen-specific CTL. In some murine tumor models, it has been clearly shown that both Th1 and Th2 can initiate a CTL-based immune response. Investigations have shown that the infusion of Th cell clones into tumor-bearing animals activated a CTL-mediated anti-tumor response [7]. Interestingly, there were no qualitative, only quantitative, differences between the Th1 and Th2, suggesting that there are multiple mechanisms boosting CTL immunity. One mechanism that has recently been described by Giuntoli and colleagues [17] involves direct interaction between Th cells and CTL. Investigators observed, in an in vitro model system, that the function of tumor-specific CTL is enhanced by Th cell through co-stimulatory molecules present on the surface of the CTL, such as CD27, CD134, and MHC class II. In addition to direct contact, Th cells can directly activate CTL through cytokines such as IL-2 which can directly stimulate growth of CTL [18]. Th cells can also enhance CTL activity indirectly by activating other cell types that can subsequently influence CTL. For example, Th1 cells release IFN-γ which activates APC to upregulate molecules such as LMP2, LMP7, MECL, PA28, and MHC class I, all of which contribute to increased antigen presentation to CTL [19]. Th1 induces the production of opsonizing antibodies that enhance the uptake of tumor cells into APC [6]. These activated APC can then directly present tumor antigen and promote expansion of tumor-specific CTL. In addition to activating and expanding CTL from the naïve T cell pool, studies also show that Th cell help is required for reactivation of memory CTL [20]. Overall, Th cells produce many factors which are able to, either directly or indirectly, influence the tumor antigen-specific CTL response.

Although the direct tumor cell-killing effects of Th cells are less well-characterized some studies suggest that these cells are able to mediate cell death through the direct contact of the Th cell with the tumor cells. Th cells can directly interact with tumor cells through MHC class II molecules. The interaction of Th cells and human tumors was initially described by Topalian and colleagues who demonstrated that melanoma T cell infiltrates contain Th cells that can directly recognize tumor cells expressing MHC class II molecules through antigen presentation [21]. This was recently shown by Perez and colleagues [22] who identified a naturally processed and presented HER-2/neu peptide epitope. The interaction between the Th cells and the tumor cell through MHC class II may impact the tumor cell in a number of ways such as through the upregulation of death-inducing receptors or the elaboration of toxic secretions. Th cells can induce the apoptosis of tumor cells through one of several related mechanisms. Th cells can use the Fas/FasL pathway to directly induce apoptosis of tumor cells. For example, Th cells directly upregulate Fas expression on the lymphoma cell surface by ligating to tumor cell CD40. Subsequent to upregulation of Fas by lymphoma cells, Th cells can directly induce tumor cell apoptosis by FasL ligation [23]. In other tumor types, there are other apoptosis mechanisms that Th cells utilize. For example, Thomas and Hersey revealed that Th cells mediate killing of melanoma and T cell lymphoma cells using a mechanism involving TNF-related apoptosis-inducing ligand (TRAIL) [24]. Th1 cells can also utilize a granzyme-perforin-dependent pathway for killing T lymphoma cells as recently shown by Echchakir and colleagues [25]. Thus, studies evaluating direct killing of tumor cells by Th cells have revealed that Th cells can have direct cytolytic effects via multiple pathways.

Initially, it was thought that the primary role of Th cells for the tumor-specific immune response was to prime and sustain CTL immunity, although some early studies alluded to a broader role of Th cells. For example, studies by Dranoff and colleagues [26] demonstrated that depletion of Th just prior to tumor challenge but following vaccine priming of CTL resulted

in loss of the ability to reject tumor, indicating a more immediate and direct role. Additional studies demonstrated that CD8-knockout mice could be immunized to reject tumors in a CD4-dependent manner [6]. The tumors used in this study were MHC class II-negative and the investigators found that both Th1 and Th2 cells were important for the maximal anti-tumor response. Th1 cells produce IFN-γ which activate tumor macrophages to produce both nitric oxide and super-oxide, both of which play an important role in tumor killing. Macrophages also may be directly involved in the IL-12-induced Th cell-dependent tumor rejection [27]. The Th2 cells could recruit and activate eosinophils that were also able to produce anti-tumor factors [6]. These findings demonstrate that Th cells mediate their anti-tumor immune responses, at least in part, through modulation of effectors of the innate immune system.

It is now becoming clear that Th cells can also be induced to have a regulatory phenotype similar to CD4+ CD25+ T regulatory cells. The induction of Th-like regulatory T cells can significantly impede the function of Th cells in the tumor microenvironment and prevent tumor eradication. Recent studies have shown the induction of Th-like regulatory T cells by co-culture with natural T regulatory cells [28]. The resulting Th-like regulatory T cells produce immunosuppressive cytokines (e.g. TGF-β) which block the function of emerging antigen-specific Th cells.

Tumor specific Th cells can mediate an anti-tumor effect via a variety of mechanisms (Fig. 1), including enhancing and supporting the immune environment via cytokine secretion, directly stimulating the recruitment of CTL, direct cytotoxic effect to the tumor itself, and orchestrating the recruitment and activation of innate immune cells. A major issue in the development of cancer vaccines designed to specifically elicit tumor specific CD4+ T cells is the definition of potential Th epitopes for candidate vaccine antigens.

Identification of T helper epitopes

The most fundamental step in the activation of Th cells relies on the interaction of T cell receptor (TCR) with tumor antigen peptide-presenting MHC-class II molecules on target cells (e.g. DC, tumor cells, etc.). MHC class II molecules are more permissive than MHC class I molecules in the length and exact amino acid sequence of bound peptide. X-ray crystallography of MHC class II molecules has revealed that the peptide binding groove is open-ended, as compared to the close-ended MHC class I [29, 30]. MHC class II molecules bind peptides with lengths ranging from 9 to 31 amino acids [29, 31, 32]. Efforts over the last decade in defining epitopes, as well as allelic differences in MHC class II, have resulted in the development of numerous physical and mathematical models that can aid in the identification of MHC class II-binding peptide ligands.

There are several models available based on which predictive algorithms can be created, including [1] position or motif-based approaches, [2] artificial neural networks (ANN), and, [3] virtual matrices. The position-

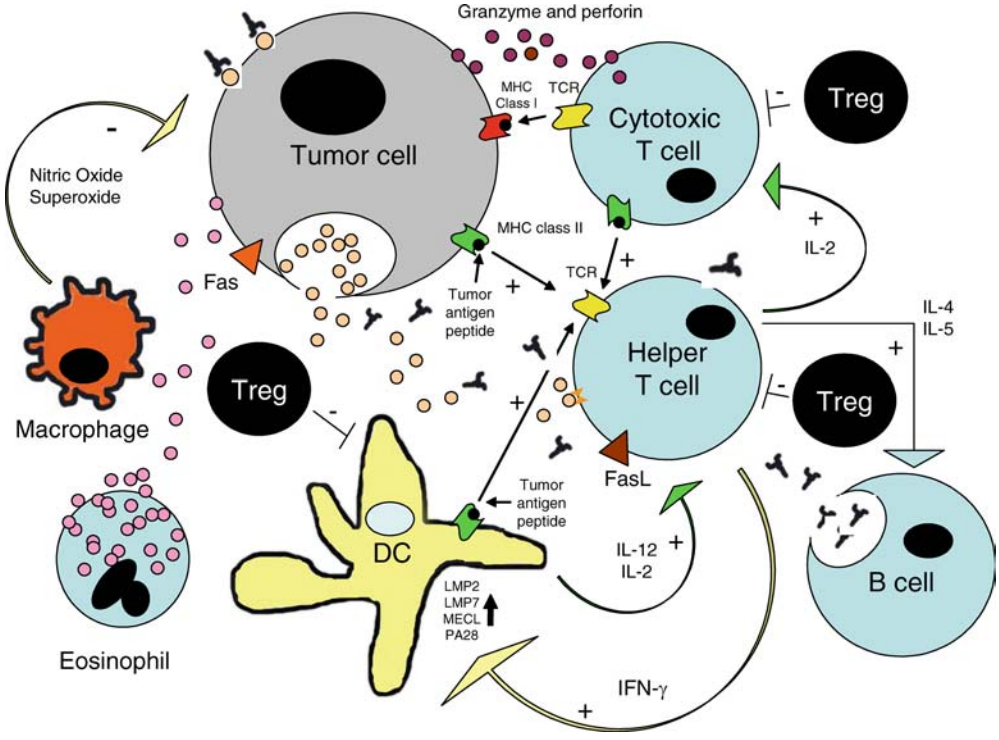

Fig. 1 The complex role of the T helper cell in the tumor microenvironment. The Th cell plays an extensive role and is able to interact with the tumor cell and a number of immune effectors through contact-dependent or contact independent mechanisms. This simplified diagram omits many other important elements of the role of the Th cell including several types of co-stimulatory cell surface molecules. A *plus sign* indicates a potentially positive impact on the antitumor response while a *negative sign* indicates a detrimental effect

based or motif-based approaches involve the identification of amino acid residues or regions (i.e. binding motifs) that are critical for anchoring the peptides into the MHC class II cleft [33]. These approaches rely on the simple primary structure and position of critical amino acids. A common predictive algorithm used, based on binding motifs, is the SYFPEITHI Database (http://syfpeithi.bmi-heidelberg.com/) [34]. An additional algorithm found useful is the motif-based algorithm called TSites (no longer readily available). TSites combines four algorithms for identifying motifs according to charge and polarity patterns and tertiary structure, particularly related to amphipathic alpha helices [35]. Each of the four searching algorithms had been successful in identifying a substantial proportion (50–70%) of helper T cell epitopes in foreign proteins. An analysis of the HER-2/neu tumor antigen, using this program, resulted in the identification of more than 40 peptides with a high potential to interact with MHC class II [36]. Several of these peptides became the basis of MHC class II peptide-based vaccines targeting HER-2/neu and were shown to be immunogenic in patients with breast and ovarian cancers [37, 38]. More recently, Southwood and colleagues developed an anchor-based algorithm for predicting degenerate peptides that are capable of binding to several common MHC class II types [39]. Such an algorithm is likely useful for constructing a multi-peptide vaccine with broad population coverage.

Artificial neural networks are self-training programs that are able to find patterns associated with large data sets. Honeyman and colleagues [40] developed an ANN to predict potentially immunogenic peptides that bind DRB1*0401 (DR4) by training it using hundreds of peptides known to bind or not to bind to DR4. The trained ANN was then tested against an array of 68 peptides, 16 amino acids in length, derived from the intracellular domain of the phosphatase PTP-IA2 [40]. In vitro binding studies revealed that the ANN had sensitivity of approximately 55% and a selectivity of nearly 90% as well as an overall accuracy of approximately 78% to predict binding epitopes. An ANN available to predict binders of HLA-DR4 and HLA-DR4Pred (http://www.imtech.res.in/raghava/hladr4pred/info.html) is available on the internet.

Virtual matrices assess the contribution to binding of all amino acids at each position within the binding core of a peptide. Virtual matrices enhance the prediction of promiscuous epitopes which can bind to multiple MHC class II alleles. Matrices are generated from ''pocket profiles'' which are the quantitative representation of the interaction of all amino acids within a single given MHC class II peptide-binding cleft [33]. A set of pocket profiles forms the virtual matrix used to determine binding epitopes. Bian and colleagues developed a virtual matrix software program called TEPITOPE. TEPITOPE has been used to predict MHC class II epitopes from numerous tumor antigens [41–43]. The program has been used to find promiscuous epitopes of MAGE3. TEPITOPE predicted 11 peptides that were subsequently tested in in vitro assays and all 11 peptides were found to bind to at least three different HLA DR alleles. Of the 11, four were naturally processed epitopes. Matrix-based algorithms can have accuracies ranging from 60% to 70% [44]. Both TEPITOPE (http://www.vaccinome.com) and an MHC class II prediction server, called ProPred, (http://www.imtech.res.in/raghava/propred/) are available on the internet. Recent data suggest that a combination of all computer-aided approaches is more likely to identify degenerate binding MHC class II peptides [45]. Preliminary analysis indicated that there is a better chance of a peptide that was identified by all three of the algorithms being an authentic HLA-DR binder than a peptide that was identified by only one or two algorithms.

Once the predicted epitopes have been identified and constructed, the next step is to determine their ability to elicit a T cell response. Strategies used to identify those peptides that have any likelihood of being natural epitopes include immunization of MHC class II transgenic mice or in vitro stimulation of T cells derived from cancer patients or normal donors. There is some in vitro evidence to suggest that the affinity of binding to MHC class II may be predictive of the immunogenicity of the peptide in vivo. For example, using competitive inhibition assays, nine HER-2/neu peptides used to immunize breast cancer patients in a clinical trial were analyzed for their binding affinity to 14 common HLA-DR molecules [46]. For 12 of the 14 DR molecules, at least one of the HER-2/neu peptides was identified to bind in vitro with high affinity. In contrast, none of the peptides bound DRB1*0802 or DRB3*0101 with high affinity. Overall, five of nine peptides exhibited high binding affinity, to three or more of the 14 DR alleles analyzed. Those peptides which exhibited high binding affinity to ≥1 DR molecules were associated with the ability to elicit an immune response in 50% or more of patients immunized with that specific HER-2/neu peptide. The percentage of DR molecules that bound with high affinity to a given peptide was highly correlated with the percentage of patients who responded to that peptide when it was administered as a vaccine. Thus, in vivo immunogenicity of MHC class II peptide epitopes may be predicted by in vitro binding affinity. However, there is some evidence to suggest that as the affinity increases, tolerizing mechanisms become a major factor in controlling the T cell response. Gross and colleagues showed that the T cell repertoire, in HLA-A2 transgenic mice, was partially tolerized to peptides that exceeded a defined affinity for MHC class I [47]. Therefore, it may be prudent to establish, in in vitro assays, that immunity can be achieved in a defined percentage ($>10\%$) of patient or normal donor peripheral blood mononuclear cells to ensure that a functional T cell receptor repertoire is available for targeting in in vivo clinical trials.

The length of the MHC class II peptide and the permissiveness of the peptide binding in the groove of the MHC molecule can also result in MHC class II peptides being promiscuous binders, thus, universal

epitopes can be identified. Several epitopes derived from common tumor antigens have been shown to elicit T cell responses across multiple MHC class II alleles. Recent investigation utilizing the program TEPITOPE identified epitopes derived from CEA that could elicit T cells in vitro from both healthy donors and cancer patients [48]. Similarly, MHC class II epitopes for hTERT, a tumor antigen upregulated widely in human tumors, have been shown to broadly bind and induce CD4+ T cell responses in vitro across several MHC class II alleles [42]. Promiscuous binding of class II epitopes have also been defined for tumor antigens NY-ESO [49], LAGE-1 [50], MAGE-3 [43], and HER-2/neu [51].

Vaccinating to augment tumor antigen-specific CD4+ T helper immunity

Immunizing patients to elicit Th immunity may be a reasonable means to generate sustained, long-lived immunity and even augment CTL responses. Vaccine strategies have largely focused on the generation of tumor antigen-specific CTL. A potential pitfall of the use of single MHC class I binding peptides is illustrated by evaluating vaccination of HER-2/neu with a well-defined HLA-A2 binding peptide p369–377 [38]. Six HLA-A2 patients with HER-2/neu-overexpressing cancers received six monthly vaccinations of 500 μg of HER-2/neu peptide, p369–377, admixed with 100 μg of GM-CSF. The patients had either stage III or IV breast or ovarian cancer. Immune responses to the p369–377 were examined using an IFN-γ ELISPOT assay. Prior to vaccination, the median precursor frequency, defined as precursors/10^6 PBMC, to p369–377 was not detectable. Following vaccination, HER-2/neu peptide-specific precursors developed to p369–377 in just two of four evaluated subjects. The responses were short-lived and not detectable at 5 months after the final vaccination. Immunocompetence was evident, as patients had detectable T cell responses to tetanus toxoid and influenza. These results demonstrate that HER-2/neu MHC class I epitopes can induce HER-2/neu peptide-specific IFN-γ-producing $CD8^+$ T cells. However, the magnitude of the responses was low and short-lived. Theoretically, the addition of $CD4^+$ T cell help would allow the generation of lasting immunity.

A more successful vaccine strategy for generating peptide-specific CTL capable of lysing tumor expressing HER-2/neu, and resulting in durable immunity, involved immunizing patients with putative T-helper epitopes of HER-2/neu which had, embedded in the natural sequence, HLA-A2 binding motifs of HER-2/neu. Thus, both CD4+ T cell help and CD8+ specific epitopes were encompassed in the same vaccine. In this trial, 19 HLA-A2 patients with HER-2/neu-overexpressing cancers received a vaccine preparation consisting of putative HER-2/neu helper peptides [52]. Contained within these sequences were the HLA-A2 binding motifs. Patients developed both HER-2/neu-specific CD4+ and CD8+ T cell responses. The level of HER-2/neu immunity was similar to that of viral and tetanus immunity. In addition, the peptide-specific T cells were able to lyse tumor. The responses were long-lived and detectable for more than 1 year after the final vaccination in selected patients. These results demonstrate that HER-2/neu MHC class II epitopes containing encompassed MHC class I epitopes are able to induce long-lasting HER-2/neu-specific IFN-γ-producing CD8 T cells. Currently, several groups are focusing on the identification of peptides that are suited for binding to MHC class II and contain, within their natural sequence, MHC class I epitopes for the same antigen. A peptide with dual MHC class I and II specificities has recently been identified for NY-ESO [53].

Stimulating an effective T helper response, even without concomitant CD8+ peptide vaccination, is a way to boost antigen-specific immunity, as CD4+ T-cells can generate the cytokine environment required to support an evolving immune response. Our group has performed clinical trials by vaccinating patients with HER-2/neu T helper peptides. Sixty-four patients with advanced stage HER-2/neu overexpressing breast, ovarian, and non-small cell lung cancer were enrolled. Thirty-eight patients finished the planned course of six immunizations [37]. Patients received 500 μg of each peptide admixed in GM-CSF. Over 90% of patients developed T cell immunity to HER-2/neu peptides and over 60% to a HER-2/neu protein domain. Thus, immunization with peptides resulted in the generation of T cells that could respond to protein processed by APC. Furthermore, after 1-year follow-up, immunity to the HER-2/neu protein persisted in over one-third of patients. Immunity elicited by active immunization with CD4+ T helper epitopes was durable. Several technological improvements are being made to enhance the immunogenicity of MHC class II vaccine approaches. Gillogly and colleagues recently developed a novel technique whereby the Ii-Key segment of the immunoregulatory Ii protein is coupled to MHC class II epitopes [54]. The Ii-Key segment catalyzes the binding of the epitope to MHC class II molecules and thereby enhances the potency of the vaccine. Another approach that has recently been developed by Lu and colleagues [55] is a helper epitope-containing Trojan vaccine. In this approach, membrane-translocating Trojan peptides are linked to the helper peptides to facilitate entry into the endoplasmic reticulum and trans-golgi network for processing and presentation by MHC class II molecules [55].

As additional CD4+ Th epitopes are being defined in other tumor antigen systems, clinical studies are being designed to assess whether immunization with CD4+ Th epitopes alone or in combination with CTL peptides is a superior use of peptide-based cancer vaccines as compared to approaches utilizing a single MHC class I peptide. Although clinical studies focus on the magnitude of the T cell response elicited after vaccination, there may be other parameters to assess the quality of

the immune response generated with active immunization. Stimulating Th1 cells capable of homing to tumor may result in modulation of the tumor microenvironment and a broadening of the tumor-specific immune response.

T helper cells modulate the tumor microenvironment

The development of peptide-based vaccines may be uniquely suited to stimulate immunity to self-antigens such as tumor antigens. The ability to mount an immune response is related to the immunodominance of specific antigenic determinants during natural immunologic processing of intact protein antigens. However, only a fraction of potential determinants in an antigen are presented in an immunodominant manner, while the remaining peptides are ignored [56]. Dominantly processed self-determinants are thought to be efficient in tolerance induction [56, 57]. However, in every self-antigen, there are sequestered determinants that are unable to induce tolerance which could be immunogenic [56]. These subdominant epitopes may trigger the threshold for T-cell activation and immune recognition if they are presented. There are several potential mechanisms by which subdominant epitopes may be presented; by virtue of a self protein being overexpressed, or by local inflammation enhancing presentation. Subdominant epitopes are more effectively presented by highly activated and efficient APC such as DC or APC markedly activated by inflammatory signals from the local immune microenvironment [57]. Antigen-specific Th1 cells, homing to tumor in the immune microenvironment, may enhance the production of inflammatory cytokines and support the elaboration of a tumor-specific immune response.

A reported finding associated with immunization against tumor antigens is the broadening of the immune response to additional tumor-related proteins not contained within a particular vaccine preparation. Spreading of the immune response to other antigens, referred to as epitope or determinant spreading, is a phenomenon first discovered in the case of autoimmune disease [58]. Epitope spreading represents the generation of an immune response to a particular portion of an immunogenic protein and then the natural spread of that immunity to other areas of the protein or even to other antigens present in the environment. Theoretically, epitope spreading represents endogenous processing of antigen at sites of inflammation initiated by a specific T cell response, or ''driver clone.'' [59]. Epitope spreading has been identified after immunization with cancer vaccines. Immunization with HER-2/neu T helper peptide-based vaccines resulted in epitope spreading within the HER-2/neu protein being observed in the majority of patients and significantly correlated with the generation of HER-2/neu protein-specific T cell immunity [37]. In addition, evaluation of the antibody immunity generated while on the trial demonstrated the development of epitope spreading to additional antigens present on the patients' tumors [60]. Despite the generation of detectable immunity against the self-protein HER-2/neu, and the development of epitope spreading, none of the patients in the studies described above developed any evidence of autoimmunity directed against tissues expressing basal levels of HER-2/neu such as skin, liver, and digestive tract epithelium.

Epitope spreading is linked with the progression of several autoimmune disorders such as systemic lupus erythematosis, insulin dependent diabetes mellitus, and multiple sclerosis [58]. Epitope spreading may be associated not only with the progression of these diseases but also with the tissue destruction observed with the pathologic progression [61]. Th cells influencing the tumor microenvironment may allow such tissue destruction in the tumor bed. The phenomenon of epitope spreading has been linked with survival benefit after immunotherapy in patients with melanoma [62].

Conclusion

The use of peptide vaccines to treat cancer is a concept that has been around for many years. Despite that, there have been very few clinical successes to date. However, during the same time period a number of significant advances have changed the way we think about peptide vaccines. First, the clinical setting in which the vaccines are used, is clearly a determining factor. Most of the early trials focused on treating established aggressive tumors. It is clear now that in the face of large tumor burden, the immune response is unable to cope due to rapid tumor growth and tumor-induced immunosuppression. Thus, in recent times, the focus has shifted to reduced disease burden including no evidence of disease as better settings for immunizations where the potential effectiveness of vaccine is to prevent tumor recurrence following adequate surgery and chemotherapy. Another possible explanation for the lack of success of earlier peptide vaccines is that most trials were designed to elicit CTL immunity rather than Th immunity. The tumor-specific Th cell is most likely a major driving force in the generation, maintenance, and probably the therapeutic effectiveness of a tumor-specific immune response. Th cells can induce anti-tumor immunity by multiple mechanisms; directly, and by stimulating additional immune effectors, both innate and adaptive. This global effect on the immune environment allows tumor-specific immunity to be enhanced via multiple pathways. Significantly, Th cells can function to broadly impact the tumor microenvironment, resulting in a more comprehensive tumor-specific immune response. The increasing interest in and recognition of the importance of Th immunity has resulted in an explosion of technology to rapidly develop immune-based therapies directed at CD4+ Th epitopes. The next few years will result in extrapolation of laboratory findings to the clinical arena, evaluating strategies for specifically augmenting the

tumor-specific Th immune response for therapeutic purpose in cancer patients.

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