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Commentary

Application of central immunologic concepts to cancer: Helping T cells and B cells become intolerant of tumors

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CD4-mediated T-cell help in the activation of $CD8^+$ T cells and B cells, through linked-recognition of antigenic determinants, is a long-standing concept foundational to our understanding of immunity (presence of help) versus tolerance (lack of help). Surprisingly, this function of $CD4^+$ T cells has not been extensively examined as a means to overcome immune tolerance of the self-antigens made by tumor cells. Hesitation to employ this powerful mechanism may be due to the potential to cause unwanted autoimmune pathology. In this issue of the *European Journal of Immunology*, Snook et al. [Eur. J. Immunol. 2014. 44: 1956–1966] identify a state of split tolerance, showing that $CD4^+$ T cells specific for a number of tumor-associated self-antigens are robustly tolerant, while their $CD8^+$ T-cell and B-cell counterparts are far less tolerant. Furthermore, the authors demonstrate that provision of linked foreign helper epitopes, such as influenza hemagglutinin, substantially enhances both $CD8^+$ T-cell and B-cell responses to tumor self-antigens without causing any overt autoimmune pathology. These findings provide a strong rationale to employ foreign helper epitopes in cancer vaccines and highlight the need to fully explore therapeutic strategies that are based on well-established immunologic concepts.

Keywords: Autoimmunity · B cells · Cancer · $CD4^+$ T cells · tolerance

See accompanying article by Snook et al.

Tumor immunotherapy has, in recent years, enjoyed a renaissance since it has begun to achieve some of its long anticipated goals in the clinical setting [1]. While the idea that the immune system could be protecting us from tumors has existed almost as long as the field of immunology itself [2], and has been argued to be part of the immune system's raison d'être [3], this concept nonetheless became unpopular for several reasons, including the seeming absence of tumor development in mice lacking an adaptive immune system; however, the concept has more recently gained traction since evidence for the immune system selection of less immunogenic tumors (tumor immunoediting). Furthermore, the striking prognostic value of the analysis of immune infiltrates in tumors has firmly established the capacity of adaptive immunity to control tumors [2, 4].

There are at least two major hurdles to overcome in efforts to generate vaccines to cancer: the generation of sufficiently strong and long-lasting tumor-specific T-cell responses that do not destroy healthy self-tissues, and the recruitment of sufficient numbers of effector T cells into tumor sites and metastases. In order to address the first issue, one approach is to take advantage of the ability of $CD4^+$ T helper cells to potently synergize with $CD8^+$ T cells, promoting their activation and memory [5]. Although much of the effort in identifying T-cell epitopes for immunization in cancer has focused on self- or modified self-antigens [6], given

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the issue of self-tolerance which is further compounded by the ability of tumors to generate tolerance to themselves, it is difficult to generate sufficient T-cell help via the (modified) self-antigen route. A strategy that has long been considered to overcome this obstacle is the addition of foreign (e.g. xeno) antigens into cancer vaccines to boost immunity [7, 8], and more recent studies have provided direct evidence that the beneficial effects of this procedure are through the provision of T-cell help [9–11]. A substantial advantage of employing foreign helper determinants physically linked to determinants recognized by $CD8^+$ T cells, rather than tumor-associated helper determinants, is that the tumor cannot use either downregulation of their own helper epitopes, or induction of tolerance against these foreign epitopes, as a means of escape.

Interestingly, it has been theorized that MHC class II-restricted T cells are likely to be more self-tolerant than MHC class I-restricted T cells or B cells [12]. It would seem an insurmountable task for our immune system to become tolerant of all of the various self-antigens throughout our body. The task would be made much simpler if extensive tolerance were only needed for T cells recognizing antigens presented on the limited number of cells that express MHC class II; expression of MHC class II is restricted to several hematopoietic lineages and endothelial cells while the vast majority of cells in the body, the various parenchymal tissue cells, generally lack expression. This concept is consistent with observations of a state approaching ignorance to some self or neo-self antigens by $CD8^+$ T cells and B cells [13–15], while $CD4^+$ T cells remain robustly tolerant [9, 13]. The generalizability of this concept remained to be determined and seemed questionable based on the possibility that the self antigens were at too low a dose to induce tolerance and other studies showing intrinsic B-cell [16] and $CD8^+$ T-cell [17] tolerance. If a relatively low level of self-tolerance in the $CD8^+$ T-cell and B-cell compartments were to prove generalizable, it would provide an even stronger rationale to expect that addition of foreign helper epitopes to cancer vaccines would allow potent $CD8^+$ T-cell and B-cell responses.

In this issue of the *European Journal of Immunology*, Snook et al. [18] test whether strong $CD4^+$ self tolerance and weaker or absent $CD8^+$ T-cell and B-cell tolerance is a generalizable principle that is widely applicable in the design of cancer vaccines. The authors refer to this state of differential tolerance as "split-tolerance," akin to the split-tolerance often seen in allogeneic bone marrow transplantation [19]. Snook et al. [18] begin by examining the response to a key target for colorectal cancer vaccines, guanylyl cyclase C (GUCY2C), using immunization with an adenovirus expressing GUCY2C alone or also expressing an MHC class II-restricted influenza hemagglutinnin helper epitope (S1) [18]. They show that $CD4^+$ T cells are tolerant of self GUCY2C but that B cells and $CD8^+$ T cells respond robustly to GUCY2C and generate $CD8^+$ T-cell memory if provided the linked S1 helper epitope [18]; these responses were prevented by $CD4^+$ T-cell depletion. As expected, in knockout mice lacking GUCY2C the $CD4^+$ T cells were not tolerant and the S1 epitope was not required in order to generate B- and T-cell responses to GUCY2C. Immunization of BALB/c mice with adenovirus containing both GUCY2C and the S1 helper epitope generated a $CD8^+$ T-cell-dependent reduction in lung metastases arising from GUCY2C-expressing CT26 colorectal cancer cells and substantially extended survival (nearly eightfold longer) compared with survival following immunization without the S1 epitope. Surprisingly, this protective immunity did not result in any detectable autoimmunity to healthy self-tissues that express GUCY2C [18] and therefore identification of the mechanisms leading to differential recruitment of effector cells to tumors as opposed to healthy host tissues warrants substantial investigation. The ability to manipulate recruitment would alleviate the potential dangers of achieving a maximal antitumor response. Perhaps most importantly, Snook et al. show that their conclusions are generalizable based on similar findings with different mouse strains and tumors/tumor antigens (e.g. melanoma and breast cancer antigens Trp2 and Her2, respectively), as well as additional helper epitopes such as the synthetic pan DR epitope known as PADRE [18]. In addition to the potential clinical utility, these studies highlight the underappreciated concept of differences in the level of self-tolerance of lymphocyte subsets to specific self-antigens.

A key conceptual feature of the T-cell help mechanism in general and employed here is that the foreign helper ($CD4^+$) and effector ($CD8^+$ and B-cell) tumor epitopes must be linked (Fig. 1), meaning that they must be presented by the same antigen-presenting cell. This occurs when the epitopes are physically linked and is analogous to B-cell responses to hapten-carrier conjugates, responses that only occur when helpers to the carrier are present and the hapten and carrier are physically linked. This essential feature of T-cell help, a feature that ensures help is not given to just any cell (i.e. it increases specificity), is likely to underlie the otherwise paradoxical finding that T-cell helpers to adenovirus do not provide effective helper epitopes for the anti-GUCY2C $CD8^+$ T-cell response. As Snook et al. [18] suggest, the timing of adenoviral antigen and GUCY2C tumor antigen expression is distinct and hence presentation of these antigens will not be linked but rather be presented by different antigen-presenting cells.

In terms of the mechanism of tumor elimination, this study supports a central role for $CD8^+$ T cells that have received adequate T-cell help. $CD4^+$ T cells have also been shown to have a potent capacity to eliminate tumor cells through perforin/granzyme B or macrophage induction [20] and they can cause substantial collateral tissue damage [21], a capacity that may be of utility in preventing immune escape of malignant cells that have downregulated tumor antigen expression. Although well known for their ability to help $CD8^+$ T cells and B cells, $CD4^+$ T cells can help each other in their activation and differentiation as seen in systems where addition of a foreign helper epitope (e.g. OVA) linked to a second antigen (e.g. HEL) increases the CD4 response to the second antigen [22, 23]. Nevertheless, in the studies of Snook et al. $CD4^+$ T-cell tolerance to GUCY2C appears to be robust and not easily overcome by additional $CD4^+$ T-cell help. However, should there exist cases where tolerance in $CD4^+$ T cells to a given self/tumor antigen is not complete, provision of foreign helper epitopes could promote their activation, allowing these $CD4^+$ T cells

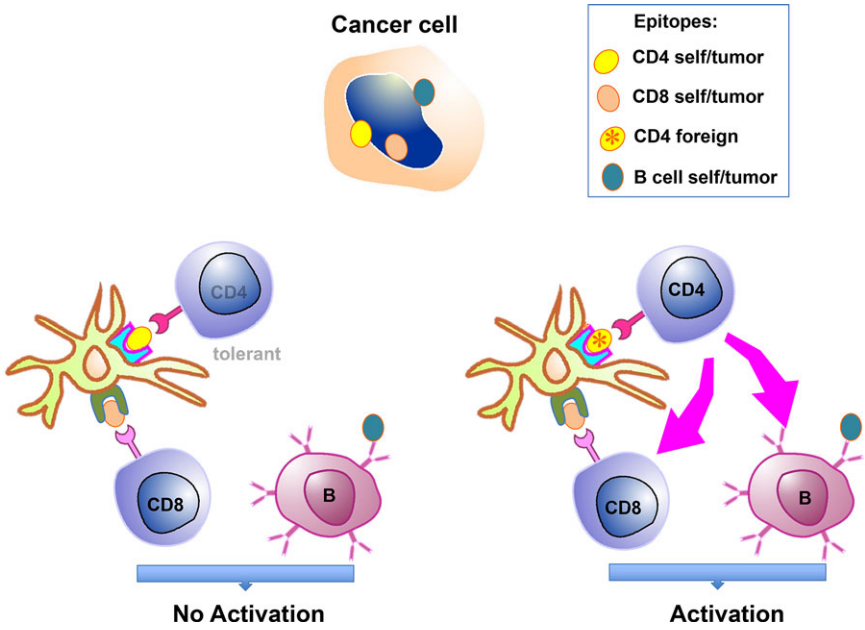

Figure 1. How split-tolerance could enhance tumor immunity. No Activation: Robust CD4$^+$ T-cell tolerance to self antigens prevents T-cell help to nontolerant CD8$^+$ T cells and B cells specific for tumor antigens. Activation: The relative lack of tolerance in the CD8$^+$ T-cell and B-cell compartments provides the opportunity to turn on tumor immunity by providing foreign helper epitopes linked to the effector epitopes recognized by CD8$^+$ T cells and B cells.

to participate in tumor elimination independent of CD8$^+$ T cells and B cells. Whether cancer vaccines should focus on the promotion of MHC class I- or MHC class II-restricted effector cells is not necessarily obvious and will require careful dissection of mechanism of tumor killing generated by the most efficacious vaccines. The benefit of CD8$^+$ T-cell responses is that they may be more self-limiting [17], causing less autoimmune damage. This, however, comes at the potential cost of allowing tumor variants to escape the effector mechanism of destruction.

Will provision of foreign helper determinants to cancer vaccines be expected to universally augment tumor immunity? The answer is likely to be no, as exemplified in a study where higher doses of a plasmid encoding a foreign helper epitope in a DNA cancer vaccine reduced vaccine efficacy and survival post tumor challenge [10]. This is consistent with the current study by Snook et al. [18], which demonstrates that additional exogenous CD4$^+$ T-cell help, when substantial numbers of specific helpers are already present (helpers to "self" GUCY2C present in GUCY2C$^{-/-}$ mice), can reduce vaccine efficacy. This might be, as the authors suggest, because these "self"-specific CD4$^+$ T cells have more anti-tumor activity independent of CD8$^+$ T cells and B cells. Alternatively, these data are predicted by the threshold hypothesis of Peter Bretscher [23], where low levels of T-cell help promote Th1 responses while high levels of T-cell help promote a Th2/antibody response at the expense of tumor-destructive cell-mediated immunity [23]. Given that T-cell help promotes different types of immunity and that Th1/CTL cell-mediated immunity is the most useful for tumor elimination, not all types or levels of T-cell help will be beneficial in tumor elimination. Examination of the IgG subclass induced in WT versus GUCY2C$^{-/-}$ mice immunized with GUCY2C-S1 may help resolve these possibilities as the subclass is directly determined by the type of T-cell helper response generated (Th1/Th2 etc.). In addition, the efficacy of a particular foreign helper epitope might not be universal. Cross-reactivity of the relevant TCR to an environmental antigen mimic might set the CD4$^+$ T-cell response to exogenous helper determinants into a regulatory/suppressive mode in some individuals. Given the above possibilities, it will be important not to abandon this well-grounded approach of linked foreign epitopes in cancer vaccines to boost the immune response should initial clinical evaluation suggest a lack of efficacy [24]. Determination of the appropriate helper epitope dose and the consequent level, type, and frequency of restimulation of T-cell help will be needed to take full advantage of this pathway. Determining these parameters for optimal exogenous T-cell help would be anticipated to contribute not only to protection against subsequent tumors but also destruction of already established tumors [25].

It is perhaps instructive that the value in tumor treatment of providing foreigner helper epitopes or blocking coinhibitors (CTLA-4 and PD-1) [26] are both direct predictions from earlier efforts to generate a broad theoretical understanding of the central problem in immunology, the self/nonself discrimination [27–29] (reviewed in [30]). Although the importance of broad theories in immunology has often been questioned [31], the current progress in tumor immunotherapy provides a testament to their value.

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