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Autoimmune complications of cancer immunotherapy

Kavita M Dhodapkar

Immunotherapy of cancer with blockade of inhibitory immune checkpoints and adoptive cell therapies have led to impressive clinical responses in several cancers. However, with increasing utilization of these therapies, immune-related adverse events have emerged as a major obstacle. Herein I discuss recent insights into the immunobiology of these toxicities. Deeper understanding of the underlying pathogenic mechanisms, cellular and molecular pathways involved, similarities and differences with spontaneous autoimmunity, and identification of clinically relevant predictive biomarkers is needed to develop optimal approaches to prevent and treat these toxicities, without compromising the therapeutic benefit from these immune therapies. These events may also provide a unique window into mechanisms underlying spontaneous autoimmunity.

Address
Emory University and Children's Healthcare of Atlanta, Atlanta, GA, United States

Corresponding author:
Dhodapkar, Kavita M (kavita.dhodapkar@emory.edu)

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The clinical challenge of immune-related adverse events (irAEs) in cancer immunotherapy

Immune-therapies such as blockade of immune checkpoints and adoptive cell therapies have begun to transform the therapeutic landscape in cancer. In particular, antibodies targeting inhibitory immune checkpoint (ICP) molecules cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), programmed death-1 (PD-1), and programmed death ligand-1 (PD-L1), either alone or in combination, can lead to durable tumor regressions and have now been approved for treatment of several different cancers. However, use of these therapies has also been associated with increase in irAEs [1,2]. This is particularly evident in the setting of combination therapy with CTLA-4 and PD-1 blockade where increase in tumor regressions following combination therapy is associated with a marked increase in Grade 3 and 4 irAEs resulting in significant morbidity and discontinuation of therapy in up to one third of patients [2,3••]. Therefore, irAEs represent a major therapeutic challenge, not only in terms of the morbidities associated with these toxicities, but also limiting the ability to administer ICPs. The pattern, severity and onset of specific irAEs seems to differ based on the specific agent, or combination [2]. For example, CTLA-4 blockade led to colitis and hypophysitis, while anti-PD-1 therapy led to higher rates of pneumonitis [4]. Combination checkpoint blockade (CCB) with anti-CTLA-4 and anti-PD-1 is associated with high rates of early and multi-organ autoimmunity, including hepatitis [3••]. These clinical events raise several questions. For example, a) to what degree the ICP-induced irAEs resemble conventional autoimmune diseases, and represent activation of an occult autoimmune state, or do they represent alternate mechanisms of auto-inflammation initiated by altered tolerance mechanisms?; b) what are the mechanisms that impact involvement of specific tissues; c) what are the key cell types that mediate these effects and do they differ from those responsible for anti-tumor effects; d) does the biology of 'early' irAEs which occur within days of starting therapy differ from those manifestations that develop over a more delayed course such as several months or years after initiating therapy; d) how can we identify patients at risk and effectively prevent such complications without impacting tumor regression? Deeper understanding of the underlying mechanisms, particularly in patients undergoing these therapies is therefore urgently needed.

Insights from animal models: opportunities and limitations

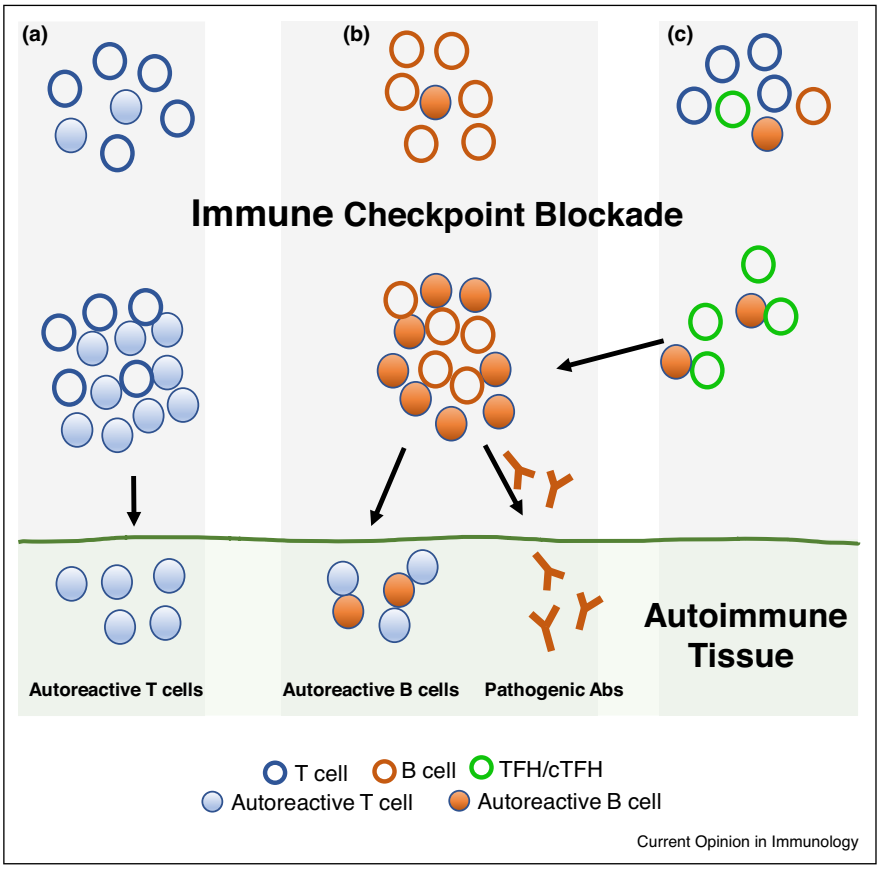
CTLA-4 deficient mice rapidly succumb to lymphoproliferative disease leading to multi-organ failure [5]. In contrast, PD-1 deficient mice have a milder phenotype with delayed and tissue-restricted autoimmunity in a strain-dependent manner [6]. Autoimmunity is also evident for mice deficient in other immune checkpoints now entering the clinic, although preclinical data have not been entirely replicated in the clinic. For example, combined loss of PD-1 and LAG-3 results in lethal autoimmunity in mice, although in early clinical experience, the safety profile of anti-LAG3 plus nivolumab was similar to that of nivolumab alone [7]. While these models were critical to the early discoveries relating to immune-regulatory function of these molecules, they also do not seem to faithfully recapitulate the patterns of irAEs observed in the clinic, such as pneumonitis in patients treated with anti-PD-1 therapy [8]. Part of the limitation may be due to the fact that many of the cancer animal models rely on B6 or BALB/c strains

which are generally resistant to autoimmune complications. Species-specific differences in biology may also represent a challenge. For example, deficiency of regulatory T cells (Tregs) is a more prominent feature of CTLA-4 blockade in mouse models than in human trials [9]. Studies in autoimmunity-prone strains may also be target-specific. For example, in autoimmune diabetes-prone non-obese diabetic (NOD) mice, anti-PD-1 rapidly induces diabetes [10], while the effects of anti-CTLA-4 are mostly restricted to newborn mice [11]. The risk of autoimmunity may also depend on the underlying genetic makeup of the individual. Recent advances in genetic humanization of mice may allow dissection of these effects by studying effects of checkpoint blockade *in vivo* utilizing human samples [12]. Nonetheless, these studies point to the unmet need to systematically study patients receiving immune checkpoint therapies with a particular emphasis on dissecting mechanisms of autoimmunity, as well the development of new models that faithfully recapitulate autoimmune toxicities of these agents in the clinic (Figure 1).

Natural autoimmunity versus irAEs

Both irAEs and natural autoimmunity can potentially share several features including autoantibodies, autoreactive T cells and underlying genetic risk factors, but may differ in terms of underlying pathogenesis, even in the setting of tissue-specific autoimmunity. Antibodies against islet-associated antigens are present in over 90% of patients with type I diabetes but in less than half of the patients who develop this as a complication of ICP blockade [13]. Evaluation of global antibody profiles in patients receiving ICP-blockade identified signatures associated with patients who developed severe irAEs [14]. Patients with spontaneous autoimmunity such as lupus have distinct molecular signatures in B cells leading to altered B cell differentiation

Figure 1



Pathways to autoimmunity following checkpoint blockade.
Several mechanisms have been proposed for induction of irAEs that involve pathogenic B or T cells. Panel **(a)**. Patients with pre-existing autoreactive T cells against antigens expressed in involved tissues may undergo activation following ICP blockade and contribute to autoimmunity; Panel **(b)**. Autoimmunity may be mediated by activation and expansion of preexisting autoreactive B cell clones such as $CD21^{lo}$ B cells. These clones and downstream plasmablasts may be responsible for the generation of pathogenic Igs and may also serve as antigen-presenting cells to activate T cells; Panel **(c)**: Activation of autoreactive B cells may depend on help provided by T_{FH} or T_{PH} cells. The antigens targeted by pathogenic B or T cells in most patients are currently unknown and in some instances they may be cross-reactive to antigens expressed on tumor cells.

and function [15,16]. It remains to be established whether identification of pre-existing autoimmune signatures will identify patients at greater risk for irAEs. Implications of underlying genetic susceptibility risks may also exist but may differ between natural autoimmunity and irAE and need further study. For example, HLA markers DQ8 and DR53 are associated with spontaneous hypophysitis but not ICP-induced hypophysitis [17]. Patients who experienced ICP-induced diabetes had a significantly higher proportion of HLA-DR4, exceeding that observed in Caucasians with spontaneous type I diabetes [13]. Certain autoimmune disease such as lupus are more common in African-American patients, but their susceptibility to ICP-induced irAEs needs further study [18]. As the development of irAEs in the clinic is a relatively recent phenomenon, long term consequences of such autoimmunity have not yet been studied. ICP-induced irAEs in principle offer the potential for fundamental insights into the early origins of autoimmunity because the biology of such early clinical phase of natural autoimmunity is very hard to study in humans. Deeper understanding of similarities (and differences) between natural and ICP-induced autoimmunity including dissection of antigenic targets and cellular mechanisms may help identify patients at increased risk for the development of irAEs, which in turn will aid optimal management of these patients.

Cellular and pathophysiologic mechanisms

Both B and T cells have been implicated in the pathogenesis of autoimmune states. Histo-pathologic analyses of involved tissues such as colitis in the context of anti-CTLA-4-induced autoimmunity was found to show enrichment of both B and T cells [19]. Humans with CTLA-4 deficiency have increased propensity for the development of autoimmunity, and exhibit alterations in both B and T cells [20]. In melanoma patients receiving combination ICP blockade, patients who experienced early changes in B cells were at increased risk for the development of autoimmune implications [21••]. These changes involved a decline in circulating B cells, concurrent with enrichment of $CD21^{lo}$ subset of B cells and plasmablasts. These changes were associated with an increase in plasma CXCL13 as a biomarker of germinal center activity [21••,22]. Early decline in B cells as well as pre-existing anti-acetylcholine receptors antibodies were also associated with high risk of irAEs in patients with thymoma treated with immune checkpoint blockade [23]. $CD21^{lo}$ double negative-2 (DN2) cells are now appreciated as a distinct subset enriched in autoreactive B cells and an increase in this B cell subset has been described in some patients with autoimmune diseases [24–30]. $CD21^{lo}$ B cells were initially thought to be anergic or exhausted B cells [26]. However, a recent study reported that these cells are potent antigen presenting cells [31]. They lack lymphoid homing chemokines and instead express chemokine receptors important for trafficking to sites of inflammation in non-lymphoid tissue [31]. $CD21^{lo}$ B cells were also recently described as new germinal center emigrants primed for plasma cell differentiation [32] that show overlap with tissue-homing innate-like B cells [33]. Recent studies in lupus patients have characterized components of the human EF B cell activation pathway including activated naïve B cells that differentiate into effector B cells (DN2 cells), representing epigenetically primed precursors of ASC [15,16,34,35•,36,37]. Together these studies point to the extrafollicular pathway as a major contributor to disease-specific autoantibodies in lupus, which is induced by TLR7 and a combination of IFNγ and IFNλ stimulation. Recent data also suggest that $CD21^{lo}$ B cells are the precise subset of human B cells specifically enriched for PD-1 expression [21••] and undergo *in vivo* activation and proliferation following CCB therapy positioning them as important targets of CCB therapy. Combination blockade in humans may therefore essentially phenocopy the features of germline CTLA4 deficient cohorts with increase in $CD21^{lo}$ B cells. Additional blockade of PD1 may then provide release of inhibition on PD1-expressing $CD21^{lo}$ B cells, ultimately leading to B cell mediated autoimmune complications. This subset of B cells is therefore an attractive candidate as a target for mediating irAEs following ICP blockade and improved characterization of this subset of pathogenic B cells may lead to novel therapeutic approaches targeting these cells.

Prior studies have also tried to correlate alterations in T cell repertoire in patients receiving ICP blockade and the risk of irAEs. For example, an increase in circulating T cell clones was linked to the risk of severe irAEs following ipilimumab therapy [38•]. In another study, immune toxicities following CTLA-4 blockade were also associated with early diversification of T cell repertoire [39]. At present, the relationship, if any, between T cells mediating anti-tumor effects versus those that might contribute to irAEs remains unclear. While T cells infiltrating or resident in tumors are emerging as key players in the context of tumor immunity [40,41], their relationship to those infiltrating organs/tissues involved in autoimmune inflammation needs to be clarified. Such studies will require tissue biopsies of involved organs as well as tumor tissue.

The antigenic targets of pathogenic antibodies causing autoimmunity or T cells infiltrating involved organs remains an area of active exploration. In particular, it would be of interest to determine if some of these antibodies also target antigens expressed on tumor cells. Another possibility is that these irAEs represent activation of clinically occult autoimmunity and involves preexisting autoreactive antibodies or T cells. Further studies of systematic dissection of antigenic targets of auto-reactive B or T cells should help clarify these issues. A recent study has shown that patients with metastatic but non-progressive tumors have persistent B cell responses expressing shared paratopes targeting public tumor antigens [42]. Another mechanism involved in the pathogenesis of irAEs may involve direct binding of the therapeutic antibody to the involved tissue. For

example, in the setting of anti-CTLA-4-associated hypophysitis, the hypophyseal cells were shown to express CTLA-4, which led to direct antibody binding [43••]. Similarly, islet cells in pancreas were shown to express PD-L1, which may permit direct binding of the antibody to these targets [44]. It is important to note that some patients may experience autoimmune manifestations involving multiple organs following ICP blockade. In this setting, several pathogenic mechanisms may coexist at the same time in different tissues. Differences in underlying pathogenic mechanisms may even apply to toxicities within the same tissue. For example, dermatologic manifestations of ICP-blockade are diverse and range from mild maculopapular rash to bullous lesions, vitiligo, lichenoid reactions and psoriatic lesions [45,46]. Differences in pathogenesis may also underlie the kinetics of irAEs and may differ between those that occur early versus those that occur many months after initiating ICP blockade. Finally, it is now well appreciated that each of the current ICP blockade strategies leads to a distinct genomic signature in humans [47]. This is also likely for many of the novel combination therapies that are currently being tested in the clinic. These differences may account for the differing rates and patterns of irAEs observed in the clinic following these therapies. In the setting of combination ICP therapy, the risk of irAE may depend more on one drug versus the other [48]. For example, the risk of early irAE following combination of anti-CTLA-4 and anti-PD-1 seems to depend more on the dose of anti-CTLA-4 than anti-PD-1.

Extrinsic factors impacting irAEs

In addition to underlying host genetics, preexisting autoreactivity or occult autoimmune disease, there is also increasing interest in the possibility that extrinsic influences may impact the risk of irAEs and thus may be a modifiable risk factor. One example of such extrinsic factors may be the nature of signals from the microbiome. In preclinical models, certain species of *Bacteroides* and *Burkholderiales* not only potentiated anti-tumor effects of CTLA-4 blockade but also reduced the risk of colitis [49,50•]. In melanoma patients receiving checkpoint blockade, the presence of *Firmicutes* species was enriched in patients experiencing colitis [51]. At present it remains to be established if the observed effect is mostly related to involved tissues (e.g. gut flora and risk of colitis) or if it impacts systemic immunity as well. The mechanisms underlying these observations remain to be clarified and may include cross-reactivity or mimicry of endogenous molecules or functional alterations in immune cells. Nonetheless, if prospectively confirmed in independent studies, alteration of microbial composition may represent a novel approach to modify risk of irAEs following ICP blockade.

Opportunities for therapeutic intervention or prevention

Studies evaluating tumor regressions in patients experiencing irAEs have yielded conflicting results, with some reporting no impact [52] while others reporting improvement in response [4]. As corticosteroids are the mainstay of therapy of these toxicities, an important consideration is whether it will also impact anti-tumor efficacy. Several studies suggest that corticosteroid usage does not seem to impair outcome. However, this does not exclude the possibility that steroids may have blunted a favorable outcome. Appreciation of dysregulation of cytokines such as tumor necrosis factor (TNF) and IL-17 has led to application of anti-cytokine approaches in the treatment of some of these toxicities [53]. Some examples include tumor necrosis factor-targeted therapies in steroid refractory colitis and the use of interleukin-6 targeted approaches for therapy of cytokine release syndrome following infusion of chimeric-antigen receptor T cells [54]. Recent insights that early changes in B cells predict the risk of autoimmune complications following combination ICP blockade [21••] have prompted initiation of randomized clinical studies specifically targeting B cells. B cells may in principle serve both a favorable and unfavorable role in tumor immunity [55,56]. Several subsets of B cells including those expressing PD1, have been shown to promote tumor progression in preclinical models [57–59]. Selective depletion of B cells in patients undergoing combination ICP blockade may therefore help dissect the role of B cells and determine if this approach can reduce irAEs and enhance antitumor efficacy. It is reassuring to note that depletion of B cells does not seem to impact anti-tumor effects of ICP blockade in preclinical models of melanoma [60]. It is however through systematic evaluation of patients undergoing irAE-targeted therapies that we will identify patient-specific biomarkers and targets to prevent these complications. These autoimmune phenomena may also provide a unique window into understanding basic mechanisms of immune tolerance and early events into the pathogenesis of spontaneous autoimmunity.

Conflict of interest statement

Nothing declared.

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