Mechanisms of Immune-Related Complications in Cancer Patients Treated with Immune Checkpoint Inhibitors

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Abstract
Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancer patients [1–4]. A recent analysis has shown that >50% of patients with advanced melanoma treated with programmed cell death protein-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blockade are still alive at 5 years [5]. More than 40% of cancer patients in the USA are currently being treated with ICI [6]. The number will increase over the next years and will also include new combination therapies [7, 8]. Compared to chemotherapy, the spectrum of side effects is clearly different and includes mainly autoimmune and autoinflammatory complications [9–12]. As shown in different clinical trials, immune-related adverse events (irAEs) are a common complication of treatment with checkpoint inhibitors. While the clinical features and treatment algorithms have been widely published and adopted as standard operating procedures in the clinics [13], the exact mechanisms of irAEs are not well understood [14, 15]. In this review, the current knowledge of mechanisms involved in immune-mediated complications is summarized and put into perspective what this could mean for the clinical management of patients treated with ICIs.

Introduction
Immune checkpoint inhibitors (ICIs) have revolutionized the treatment of cancer patients [1–4]. A recent analysis has shown that >50% of patients with advanced melanoma treated with programmed cell death protein-1 (PD-1) and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) blockade are still alive at 5 years [5]. More than 40% of cancer patients in the USA are currently being treated with ICI [6]. The number will increase over the next years and will also include new combination therapies [7, 8]. Compared to chemotherapy, the spectrum of side effects is clearly different and includes mainly autoimmune and autoinflammatory complications [9–12]. As shown in different clinical trials, immune-related adverse events (irAEs) are a common complication of treatment with checkpoint inhibitors. While the clinical features and treatment algorithms have been widely published and adopted as standard operating procedures in the clinics [13], the exact mechanisms of irAEs are not well understood [14, 15]. In this review, the current knowledge of mechanisms involved in immune-mediated complications is summarized and put into perspective what this could mean for the clinical management of patients treated with ICIs.
Immune Checkpoint Inhibitors

ICIs are monoclonal antibodies that interact with the regulation of checks and balances between immune cells and tumor cells. They exploit the capacity of the immune system to protect against cancers by T cells targeting tumor antigens [7, 16, 17]. ICIs currently used in clinical practice are monoclonal antibodies blocking the inhibitory receptors cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or PD-1 or its ligand programmed cell death ligand-1 (PD-L1) [7, 16, 17]. As our immune system can differentiate between self and nonself, ICIs work mainly in cancer types being recognized as nonself with an increased foreignness, that is, cancers with a high amount of mutations [18]. In some cancer types, for example, melanoma, non-small-cell lung cancer (NSCLC), and urothelial and renal cell carcinomas, ICIs have demonstrated a high response rate and ICIs have been granted regulatory approval for different indications [7, 16, 17]. Checkpoint blockade by ICIs enables an effective antitumor activity in some patients, although many pathways of primary and secondary resistance to current ICIs have been described [19, 20].

Mechanism of Action of ICIs

Our immune system has been set up to differentiate between self and nonself [18]. The delicate regulation of immune activation toward pathogenic nonself is supported by an array of activating and inhibitory receptors. Immune checkpoints are inhibitory receptors regulating overshooting immune reactions after immune activation and limit immune-mediated destruction of our own tissue [7, 17]. Current ICIs target immune checkpoints on T cells that leads to reactivation of a tumor-specific T cell response [21].

CTLA-4 is expressed on activated T cells and constitutively on regulatory T cells (Tregs). T cell activation leads to an induction of CTLA-4 on the activated T cells. The binding of CTLA-4 to CD80/86 on antigen-presenting cells (APC) transmits a co-inhibitory signal to the T cell, leading to downregulation of the immune response. CTLA-4 has a higher binding affinity to CD80/86 than to CD28. Antibodies against CTLA-4 (CTLA-4 inhibitors) therefore counteract the inhibition of the immune response. Furthermore, CTLA-4 blocking antibodies can reduce the number Tregs, often located in the tumor microenvironment.

Another co-inhibitory signal results from binding of PD-1 on peripheral T cells to PD-L1 or PD-L2 on APC. PD-1 expression is induced by activation of peripheral T cells and B cells. Its function seems to be maintenance of peripheral tolerance [22]. Following an immune response, almost all immunocompetent cells induce the expression of PD-L1, and also many cancer cells express PD-L1 on their surface. Activated T cells recognize with their T cell receptor (TCR) tumor antigen presented by the major histocompatibility complex (MHC) and act upon the tumor cells by destroying them. This process can be downregulated by binding of PD-L1 on tumor cells to PD-1 on immune cells (immune evasion). With the administration of antibodies against PD-1 or PD-L1 (PD-1/PD-L1 inhibitors), the negative regulation of the immune response is unlocked [23].

Downstream Signaling Pathways of PD-1 and CTLA-4

Two signals from APCs are required for an effective activation of T cells (antigen recognition through interaction antigen/MHC with TCR and co-stimulatory signal). Upon interaction of antigenic peptide/MCH with the TCR, oligomerization of TCR/CD3 chains results in phosphorylation of the intracellular domains (tyrosine motifs) and recruitment of activated LCK and ZAP-70. This leads to the initiation of the downstream TCR signaling events.

PD-1 Pathway

PD-1 is a transmembrane protein with an extracellular domain, a transmembrane part, and a cytoplasmic domain with 2 tyrosine motifs: immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif. As concisely outlined in a review by Sharpe and Pauken [24], upon binding, PD-1 is phosphorylated at immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif, resulting in the recruitment of protein tyrosine phosphatases, such as Src homology 2 domain-containing phosphatase (SHP) 1 and SHP2, at these motifs [25, 26]. SHP2 is considered the main driver of PD-1 inhibitory function. Protein tyrosine phosphatases can dephosphorylate at immunoreceptor tyrosine-based inhibitory motif and immunoreceptor tyrosine-based switch motif, resulting in the recruitment of protein tyrosine phosphatases, such as Src homology 2 domain-containing phosphatase (SHP) 1 and SHP2, at these motifs [25, 26]. SHP2 is considered the main driver of PD-1 inhibitory function. Protein tyrosine phosphatases can dephosphorylate kinases, such as ZAP, countering signals that occur via TCR and CD28, and thus interrupting downstream signaling cascades including PI3K/AKT and RAS/MEK/ERK. This leads to reduced T cell activation, proliferation, metabolism and differentiation, survival, and cytokine production.
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CTLA-4 Pathway

The mechanisms induced by CTLA-4 on T cells include cell intrinsic and extrinsic pathways. As outlined by Brunner-Weinzierl and Rudd [27], intrinsic events consist of the inhibition of protein translation, recruitment of phosphatases, activation of ubiquitin ligases, inhibition of cytokine receptor signaling, and inhibition of lipid microdomain formation on the surface of T cells, while extrinsic events include the competition for CD28 in id microdomain formation on the surface of T cells, while inhibition of cytokine receptor signaling, and inhibition of lip-...
Dermatological Toxicities

Dermatological toxicities are among the most common side effects reported with ICI therapy, occurring in 30–40% of patients treated with PD-1/PD-L1 blockade and in approximately 50% of patients treated with CTLA-4 inhibitors [13]. In general, the severity of dermatological adverse events is mild to moderate (grade 1–2). For CTLA-4 inhibitors, a dose-dependent incidence rate has been described, ranging from 12.5% of any-grade toxicities with 0.3 mg/kg ipilimumab, to 47–63% with 10 mg/kg (2.4–8.3% grade 3–4 events) [36, 46–48]. The occurrence of dermatological toxicities is early in the course of treatment, and symptoms can appear even after the first dose. The most common presentation is rash and pruritus, but also serious toxicities such as Stevens-Johnson syndrome and toxic epidermal necrolysis or drug reaction with eosinophilia and systemic symptoms have been described [49–51]. Vitiligo has only been reported in melanoma patients and surprisingly has been associated with favorable treatment outcome [52]. Four histopathological groups of dermatological toxicities have been reported: inflammatory, immunobullous, alterations of keratinocytes, and alterations of melanocytes [53]. While this classification takes into account the cell type, a better classification would differentiate rather between acute and chronic affection of the skin. Chronic dermatitis is sometimes found in the form of psoriatic alterations with plaques and ICIs can be often continued while lesions are treated topically [49].

Gastrointestinal Toxicities

Colitis is one of the most frequent complication of ICI therapy, especially with CTLA-4 inhibitors and combined regimen [54]. Diarrhea is the predominant symptom of colitis. Other symptoms include abdominal pain, rectal bleeding, nausea, and emesis. Severe toxicities such as intestinal perforation and toxic megacolon can develop. There is a wide spectrum of presentations on endoscopy [55]. Importantly, grade of diarrhea seems not to be associated with endoscopic inflammation or grade of colitis [55]. Fecal lactoferrin and calprotectin have been described to be sensitive noninvasive markers of colitis [56]. The occurrence of high-grade colitis is associated with better antitumor efficacy [55]. Colitis may recur after discontinuation of therapy, and patients may present with a condition similar to chronic inflammatory bowel disease [57].

Hepatitis

Patients with immune-related hepatitis are asymptomatic or mildly symptomatic and present with elevated alanine aminotransferase and/or aspartate aminotransferase, with or without increased bilirubin [58]. Median onset of transaminase elevation has been observed between 5 and 7 weeks after starting immunotherapy [58, 59]. The incidence of immune-mediated hepatotoxicity in patients treated with ipilimumab is 4–11% [60], but depends on the dose [48], whereas the frequency is much lower with PD-1/PD-L1 inhibitors [60]. Histological assessment can distinguish a different pattern between PD-1/PD-L1- and CTLA-4-induced toxicity [59].

Endocrine Toxicities

Endocrine irAEs are among the most frequent complications associated with ICI therapy and include thyroid disorders (hypothyroidism and hyperthyroidism), hypophysitis, adrenal insufficiency, and type 1 diabetes mel-
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Pneumonitis

The overall incidence of pneumonitis is low, but the occurrence is potentially life-threatening. Pneumonitis is one of the most frequent causes of death due to PD-1/PD-L1 inhibitors [42]. Median time to onset of pneumonitis has been reported to occur at 2.8 months, with a wide range from a few days to 19.2 months [67]. The risk for pneumonitis is higher with PD-1 inhibitors than with CTLA-4 inhibitors. The incidence with PD-1/PD-L1 inhibitors is 5% for any-grade toxicities and 1–2% for grade 3–4 adverse events [67]. Frequency and severity differ for the specific treatment setting, with higher incidence and more severe reactions in patients with NSCLC and renal cell carcinoma than in melanoma patients [68]. This difference can be partly explained by the fact that patients with lung cancer often have underlying lung pathology, such as chronic obstructive pulmonary disease and lung fibrosis. The presentation of pneumonitis may vary, ranging from incidental findings on imaging studies to severe symptoms (shortness of breath and desaturation). Imaging findings may be classified into 5 subtypes according to their radiological features: cryptogenic organizing pneumonia-like, ground glass opacities-, interstitial-, hypersensitivity-type, and pneumonitis not otherwise specified, although there seems to be no correlation to survival [67]. Nearly all grade 1 and 2 pneumonitis resolve with corticosteroids, whereas grade 3/4 pneumonitis often require additional immunosuppressive agents [67].

Treatment

Different general and organ-specific guidelines have been published, addressing the management of irAEs [10, 13, 69]. These guidelines reflect general consensus agreements and clinical experiences. Unfortunately, there are only few studies that have assessed prospectively the optimal management of irAEs.

In general, upon occurrence of irAEs, immediate workup is required and action should be undertaken to prevent aggravation of irAEs. Depending on the severity, ICIs should be discontinued and immunosuppressive or immune-modulating agent treatment, including high-dose corticosteroids, tumor necrosis factor α inhibitors, mycophenolate, or tacrolimus, should be initiated to halt...
the toxicities induced by ICI treatment. Rapid onset of action is important. The mainstay of immunosuppressive therapy is corticosteroids. Initial dosing should be high, followed by careful tapering of immunosuppression.

Recurrence of irAEs after Re-exposure
The risk of re-exposing patients to ICIs, in particular, anti-PD-1/ PD-L1 agents, has high clinical relevance. Most of irAEs are well treatable and can be controlled. In one small analysis, patients with immune-related colitis due to ICI therapy were re-exposed after recovery of colitis [70]. The chance to experience colitis if re-exposure was comprising a CTLA-4 blocking agent was high and ranged between 29 and 88% [70]. In another study of 80 patients treated with anti-CTLA-4 and anti-PD-1 blocking antibodies, irAEs re-occurred in only 14% of patients re-exposed to PD-1 blocking agents [71]. In a cohort study, approximately 30% of irAEs upon rechallenge were the same as the primary manifestation [72]. During the last years, we have gained our own experience in re-exposing patients to ICIs after irAEs, and we feel confident in reusing PD-1/PD-L1 blocking agents in many cases.

Biomarkers to Predict irAEs
Reliable clinical or molecular biomarkers to predict patients at risk have not yet been identified [43]. Even conditions such as pre-existing autoimmune disorders are no absolute predictive factors for development of

**Fig. 2.** Overview of potential mechanisms involved in immune-related adverse events. **a** A proposed mechanism is a T cell reaction to shared antigens that are expressed both in tumors and inflammatory lesions. For example, this has been shown to be the case in patients with myocarditis. **b** Cases with the development of auto-antibodies have been described, for example, in cases of thyroiditis. **c** In some instances, direct binding of ICI antibodies to target organs has been proposed. For example, CTLA-4 is expressed on cells of the pituitary gland and direct immune activation by binding of CTLA-4-targeted antibodies. **d** Overactivation of innate and adaptive immune cells by treatment with ICIs could lead to an overshooting cytokine secretion and to inflammatory complications.
irAEs [73, 74]. Parameters such as baseline circulating cytokine levels, including IL-6 or IL-17 [75–77], and chemokines [78] and their dynamics upon treatment with ICIs may reflect pre-existing inflammatory conditions as explained below. PD-L1 expression is associated often with better outcome as are some irAEs [52]. However, PD-L1 expression on tumor cells and the risk for the development of irAEs require further studies. In a recent trial, the function of microRNA-146a as immunoregulator was described [79]. In a murine model, reduced levels of microRNA-146a resulted in more irAEs. Interestingly, decreased microRNA-146a expression in patients with a single nucleotide polymorphism rs2910164 was associated with an increased risk of developing severe irAEs.

Mechanisms Involved in the Pathogenesis of irAEs

The precise pathophysiological mechanism of autoimmune-mediated adverse events has not fully been uncovered. However, it seems likely that the pathogenesis follows the mechanism of action of checkpoint inhibitors, in all its complexity. Only a few studies have been published in this field giving insights into the pathophysiology of immune-related complications in patients treated with ICIs.

Generally, the following aspects can be emphasized: CTLA-4 and PD-1/PD-L1 inhibitors display a distinct pattern of irAEs, reflecting a different pathophysiological mechanism. The sequence of treatment might influence the occurrence of irAEs as well. The pathogenesis of irAEs is likely to be multifactorial.

Among different factors, genetic predisposition might play a role. MHC genes (human leukocyte antigens) are known as factors that represent a risk for the development of many spontaneously occurring autoimmune diseases [80]. In patients with ICI-induced diabetes, a predominance of HLA-DR4 was observed with a frequency significantly higher than in the general population or patients with spontaneous type 1 diabetes [81].

Several studies point toward a link between occurrence of irAEs and tumor response [82–84], leading to the conclusion that the occurrence of immune-mediated toxicities is indicative not only for an activated immune system, but also a favorable tumor immune response. Thus, onset of irAEs may be considered a clinical biomarker for response of ICIs. However, some toxicities develop without any benefit in terms of treatment response. This may be the case in the case of pre-existing inflammation (e.g., clinically not active autoimmune disease) exacerbated by the start of immunotherapy.

Here, we summarize possible causal and/or associated factors as well as potential pathophysiological mechanisms involved in the development of irAEs. We differentiate the following mechanisms (Fig. 2):

- T-cell-mediated mechanisms
- B-cell-mediated effects
- CTLA-4 expression in tissue in the particular case of the pituitary gland
- Inflammation and cytokine-mediated mechanisms
- Role of the gut microbiome

T-Cell-Mediated Mechanisms

T-cell-mediated mechanisms might be the predominant factor of irAEs. The antigen specificity of T-cell-mediated irAEs is generally unknown. However, some efforts have been made to identify antigens, and studies identifying shared antigens in tumors and inflammatory lesions have helped to achieve some progress [85–87].

Increased T Cell Diversity

Most data on the mechanism of irAEs derive from studies with CTLA-4 inhibitors. Some studies have postulated an increase in IL-17-producing T cells [88] as explanation for toxicities from CTLA-4 inhibitor treatment, reflecting also the observation that IL-17 is a mediator of colitis. Contrary to the concept of clonal T cell expansion, studies by Antoni Ribas and colleagues [89] evidenced that CTLA-4 blockade leads to an increased diversity of T cells without any specific T cell expansion. Treatment with CTLA-4 inhibitor (tremelimumab) in patients with metastatic melanoma led to an expansion of the total number of unique productively rearranged TCR V-beta complementarity determining region (CDR3) sequences in the blood. Interestingly, patients were more likely to develop toxicities if they expanded the number of unique productive sequences, which may reflect the expansion of autoreactive T cells. Another study [90] investigated patients with metastatic prostate cancer treated with ipilimumab and found that patients who developed irAEs displayed a greater T cell diversification. Taken together, these data suggest that alteration (e.g., diversification) of the T cell repertoire induced by ICI treatment (CTLA-4 inhibitor) may expand subgroups of autoreactive T cells.

Shared Antigen (Epitope Spreading and Cross-Reactivity of Self- and Tumor Antigen)

Some studies have suggested that shared antigen in the tumor and the inflammatory lesion could be an underly-
ing mechanism of T-cell-mediated toxicity. This concept alludes to the pathogenesis of rheumatic diseases, by suggesting that activated T cells target both normal and tumor tissue, leading to anti-tumor response, but also to ICI-related toxicity. This mechanism was hypothesized in a case report of 2 patients with fatal myocarditis after treatment with ipilimumab and nivolumab for advanced melanoma [85]. Autopsy of these patients revealed myocardial T cell and macrophage infiltration. Receptor sequencing of the infiltrating T cells evidenced common high frequency TCRs in the tumor, myocardial and skeletal muscle, indicating identical selective clonal T cell populations at these sites. In another study, a similar observation was made in patients with pulmonary irAEs [87]. In 4 patients, there was a significant overlap of T cell clonotypes in tumors and inflammatory lesions [87]. A prospective cohort study of patients with metastatic NSCLC treated with PD-1 inhibitors further highlights the concept of shared antigen [86]. Among these patients, 34.2% developed skin toxicities. IrAEs were more frequent in good responders to therapy (PR and CR). Analysis of patient-matched biopsies from tumor and affected skin lesions discovered identical TCR sequences, indicating an infiltration by the same T cell clones. Further analysis identified 9 potential shared T cell antigens. These antigens were able to provoke IFN-γ-positive T cell response (CD8+). IFN-γ-positive T cells were then isolated from stimulated peripheral blood mononuclear cells and compared with T cells (using TCR clonotype analyses) from tumor tissue and the affected skin. Interestingly, identical antigen-specific T cells were found, partly even in all 3 compartments. Another possible example of cross-reactivity of self- and tumor antigen is the occurrence of vitiligo in patients with melanoma responding to immunotherapy [91]. And again, there is an association between tumor response and development of immune-mediated side effect [92]. In summary, these findings indicate that specific autoimmune T cell clones recognize shared antigens in normal and tumor tissues, leading to irAEs.

**Imbalance between Tregs and T Effector Cells**

An altered balance between Tregs (Foxp3+ CD25+ CD4+) and T effector cells has been postulated to result in a loss of peripheral tolerance, leading to the development of irAEs. By inactivating autoreactive T cells, and therefore assuring peripheral tolerance, Tregs have a crucial role in the perseverance of immune homeostasis [93]. The importance of Tregs is supported by the observation that patients with loss-of-function mutations in the Foxp3 gene display severe autoimmunity [94]. A similar phenotype has been described in mouse models, in which depletion of Tregs resulted in multiorgan autoimmunity [93]. In the tumor microenvironment, Tregs induce an immunosuppressive state leading to immune evasion of cancer cells [95]. Thus, Tregs are considered important therapeutic targets in cancer immunotherapy [15]. As PD-1 and CTLA-4 are expressed on the surface of Tregs, ICIs could directly target Tregs. Among the several surface molecules expressed by Tregs, CTLA-4 is key molecule. CTLA-4 is constitutively expressed on naive Tregs, but at higher level by effector Tregs and even higher on tumor-infiltrating Tregs. Depletion of Tregs induced by ICIs in particular CTLA-4-targeting antibodies might lead to a loss of peripheral tolerance and induction of irAEs.

**B-Cell-/Antibody-Mediated Mechanisms**

Besides the predominant role of T cells in the pathogenesis of irAEs, some effects by direct or indirect action may also be attributed to B-cell- and antibody-mediated complications of checkpoint blockade. Recent evidence suggests that B cells are an important factor of ICI efficacy and the formation of tertiary follicular structures [96–98]. It is therefore likely that future investigations will uncover additional roles of B cells in the pathogenesis of irAEs.

**Direct Activation of B Cells**

A study by Das et al. [99] demonstrated that combined checkpoint blockade (CTLA-4/PD-1 inhibitor) in patients with metastatic melanoma resulted in early changes in B cells, including a decline in circulating B cells with an increase in the CD21lo subset of B cells and plasmablasts. This was not the case with PD-1 or CTLA-4 inhibitor monotherapy. The observation of an increase in CD21lo B cells has also been made in CTLA-deficient individuals [100]. In the study by Das et al. [99], the magnitude of early B cell decline correlated with the time to toxicity onset and the grade of irAEs. Treatment-induced B cell change (integrating the factors: B cell decline and increase in CD21lo B cells and plasmablasts) was predictive for the development of irAEs. Patients with B cell changes induced by combined checkpoint blockade tended to have more frequently multiple irAEs. The sample size in this study was small. The exact impact of action of checkpoint blockade on B cells, whether unfolding a direct or an indirect action (via T cells), remains unclear. However, restriction of PD-1 expression to the subgroup of CD21lo B cells (in the above-mentioned study) might hint on a direct mechanism of action.
Mechanisms of Immune-Related Complications

Presence of Autoantibodies

B-cell-mediated mechanisms seem more apparent in conditions similar to conventional forms of autoimmunity, postulating a pathogenesis that applies to both conditions (antibody presentation, autoreactive T cells, and genetic risk factors). Indeed, autoantibodies have been detected in many patients who developed irAEs similar to situations observed in patients without ICI treatment, for example, in patients with myasthenia gravis [101], thyroiditis [102, 103], autoimmune hemolytic anemia [104], small-vessel vasculitis [105], and type 1 diabetes mellitus [81]. However, comparing autoantibody positivity for islet-associated antigens in patients with conventional and ICI-induced diabetes displays a different picture. While in the former situation, islet-associated antigens are present in almost all patients, they are found in fewer than half of the cases in patients with ICI-related diabetes mellitus [81]. Similarly, in another study, only few patients with rheumatological irAEs were positive for rheumatoid factor and anti-cyclic citrullinated peptide antibodies [65]. Elevated levels of antithyroxine oxidase antibody and/or antithyrotropin receptor antibody (TgAb) have been found in many cases of ICI-induced thyroid disorders [102, 103]. But it remains unclear whether thyroid autoantibodies play a causative role in the pathogenesis and whether elevated levels at baseline increase the risk for development of ICI-induced thyroid disorders. Thyroid autoantibodies may be present at baseline or develop during treatment [103, 106]. A study by Maekura et al. [106] suggests that patients with baseline autoantibodies may be at risk of developing ICI-related thyroid disorders. Furthermore, patients who developed autoantibodies under PD-1 inhibitor treatment were associated with a higher risk of developing ICI-induced thyroid disorders [103]. Anti-PD1 treatment subsequent to ipilimumab therapy in patients who expressed thyroid antibodies led to a higher percentage of thyroid dysfunction than in patients who did not develop autoantibodies [107].

An association, but nonsignificant, between the development of any autoantibody and irAEs has been shown in patients undergoing ipilimumab treatment for metastatic melanoma [107]. Around 79% of patients who developed any autoantibody had an irAE, compared with 57.5% of the patients who did not develop any autoantibody. However, in this study, the appearance of a specific autoantibody was not associated with the occurrence of an irAE in the organ system affected by the disease for which the specific autoantibody has diagnostic value. In summary, the exact role of autoantibodies in the pathogenesis of irAEs remains unclear.

CTLA-4 Expression in Pituitary Gland

Ipilimumab-induced hypophysitis has been related to antibody-dependent T-cell-mediated cytotoxicity and complement activation [108]. Interestingly, studies have demonstrated that CTLA-4 is expressed on normal pituitary gland cells, especially in pituitary thyrotroph and lactotroph cells [109]. Hypophysitis may therefore be also a result from direct targeting of CTLA-4 antigen in the pituitary gland, leading to mononuclear cell infiltration of the pituitary gland, building of pituitary antibodies and complement activation (inflammation and ultimately destruction of the gland) [109].

Inflammation and Cytokine-Mediated Mechanisms

Inflammation and uncontrolled cytokine release as a possible explanation for the development of irAEs might be a consequence of a T-cell- and/or B-cell-mediated immune response or may itself be an independent causative factor. As mentioned above, there is an association between pre-existing circulating cytokines such as IL-17 levels and irAEs in patients with advanced melanoma under ipilimumab treatment [76, 88]. A similar observation was made in a study by Lim and colleagues [77]. Patients with metastatic melanoma experiencing severe irAEs following CTLA-4/PD-1 inhibitor combined checkpoint blockade had elevated levels of pro-inflammatory cytokines in the blood. An 11-cytokine signature prior to and early during treatment correlated with the occurrence of severe irAEs [77]. Some of the described cytokines are implicated in the inflammatory process underlying autoimmune disease. The observation of pre-existing elevated cytokine levels supports the hypothesis that irAEs represent an expansion of subclinical inflammation by ICI. Retrospective data indicate that patients treated with ICIs and a special nutritional state display an increased risk for the occurrence of irAEs [110]. For example, Deng and colleagues [111] have shown in a study of 84 ICI-treated patients that a higher BMI (≥25 kg/m^2) is associated with a significant increased risk to develop irAEs, probably due to different cytokine profile in overweight patients.

Reference to constitutive or pre-existing conditions as possible causes for the occurrence of immune-mediated side effects is provided by studies on risk factors and retrospective data of patients with autoimmune diseases. Patients with pre-existing autoimmune diseases were suspected to have an enhanced risk of developing immune-mediated side effects and were therefore excluded from most studies with ICIs. But different retrospective studies have found that although autoimmunity is often exacerbated by ICI, the symptoms are often manageable, and
the activity of treatment comparable to that in the general population [112, 113].

In summary, whereas there is a correlation between occurrence of irAEs and presence of pro-inflammatory cytokines, it is difficult to interpret and their pathogenetic or/and correlative role in the pathogenesis of immune-related toxicities requires further investigations.

Role of the Microbiome in Immune-Related Complications

The gut mucosal immune system has a prominent role in the regulation of the immune response and is thereby a site for induction of Tregs [114]. Increasing evidence suggests that the gut microbiome may play an important role in immune homeostasis and tolerance [115]. Different studies have demonstrated an association between the composition of the gut microbiota and outcome of immunotherapy in melanoma patients [116–119]. Other studies have underlined these findings and shown a significant association between specific types of bacteria and anti-tumor response of immunotherapy [117, 120, 121]. A prospective study [122] in patients with metastatic melanoma treated with ipilimumab indicated that patients whose baseline microbiota was enriched with Faecalibacterium genus and other Firmicutes had better outcome (longer PFS and OS). Interestingly, the occurrence of colitis was linked to Firmicutes, whereas no colitis-related phylotypes were assigned to Bacteroidetes. A lower level of peripheral Tregs was associated with colitis, better outcome, and baseline gut microbiome enriched with Firmicutes. In this context, the role of gut mucosal IL-17-producing T helper (Th17) cells was highlighted. Th17 cells have been implicated in the pathogenesis of many inflammatory and autoimmune diseases [123]. As mentioned above, patients with metastatic melanoma treated with CTLA-4 inhibitor tremelimumab had an increased number of circulating Th17 cells and expansion of these cells was associated with colitis [88]. The induction of Th17 cells in germ-free mice has been shown by a cocktail of pathogen-free, gut commensal bacteria [124]. The hypothesis is that the differentiation and activation of Th17 cells seem to be dependent on the activation of a subset of lamina propria dendritic cells, known as DC17 cells. These in turn are activated by bacterial products, especially ATP. Cytokines secreted by DC17 cells play a crucial role in the differentiation of Th17 cells. Th17 cells promote both anti-tumor immunity and irAEs [118]. Differentiation of Th17 cells may be counteracted by Tregs, whose activity is decreased by ICIs. Interestingly, a recent study demonstrated that fecal microbiota transplantation was able to successfully treat ICI-associated colitis [125]. This treatment led to a relative increase in the proportion of Tregs within the colonic mucosa. Resuming these data, microbiome composition may be connected with both response and development of toxicity.

Conclusion and Outlook

The presence of irAEs is indicative of an activated immune response and associations between anti-tumor immune activation and irAEs are observed. Currently, no definite pathomechanism has been established and probably various different mechanisms are involved depending on the pathway targeted (CTLA-4 vs. PD-1/PD-L1) and the cancer types treated. Further investigations are needed to delineate specific targets to interfere with irAEs, while not inhibiting the anti-tumor efficacy of ICIs. To this end, it will be important to also establish preclinical models, in which we will be able to test different mechanisms and treatments. Moreover, further translational analysis is needed of patients treated with ICI in order to establish biomarkers to predict treatment options and also to provide guidance how irAEs have to be treated.

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D.K. and H.L. have written the manuscript. All authors have approved the final version for publication.
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