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Treatment Failure of a TLR-7 Agonist Occurs Due to Self-Regulation of Acute Inflammation and Can Be Overcome by IL-10 Blockade

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Multiple TLR agonists have been shown to have antitumor effects in animal models. However, the therapeutic efficacy of TLR agonist monotherapy in cancer treatment has been limited, and the mechanisms of failure remain unknown. We demonstrate that topical treatment with a TLR-7 agonist, imiquimod, can elicit significant regression of spontaneous breast cancers in neu transgenic mice, a model of human HER-2/neu breast cancer. However, tumor growth progressed once imiquimod therapy was ended. Gene expression analysis using tumor-derived RNA demonstrated that imiquimod induced high levels of IL-10 in addition to TNF-α and IFN-γ. Elevated levels of circulating IL-10 were also detected in sera from imiquimod-treated mice. Elevated serum IL-10 appeared to be derived from IL-10 and dual cytokine secreting (IFN-γ+ and IL-10+) CD4+ T cells rather than CD4+CD25+Foxp3+ T regulatory cells, which were also induced by imiquimod treatment. Blockade of IL-10, but not TGF-β, enhanced the antitumor effect of imiquimod by significantly prolonging survival in treated mice. These data suggest that the excessive inflammation induced by TLR agonists may result in a self-regulatory immunosuppression via IL-10 induction and that blocking IL-10 could enhance the therapeutic efficacy of these agents. The Journal of Immunology, 2010, 184: 5360–5367.

The use of TLR agonists as novel agents in cancer treatment has been extensively studied over the last several years in both the laboratory and the clinic. Activating the immune system via TLR can result in the generation of both innate and adaptive immune responses, either of which have the potential to significantly impact tumor growth. Presumably, adaptive immunity stimulated via TLR-activated APCs would control systemic metastatic disease. Although TLR ligation has been shown to result in a marked systemic or local inflammatory response when used as monotherapy (1, 2) such treatment has yet to be associated with significant clinical responses in most human malignancies.

One of the most commonly used TLR agonists is imiquimod—a TLR-7 specific agent, which was originally approved by the U.S. Food and Drug Administration for the treatment of genital warts. The antitumor effects of imiquimod have been shown in multiple animal models (3–6). Although antiangiogenesis and apoptosis induction have also been suggested as the mechanism of the antitumor action of imiquimod, its major mechanism is believed to be mediated via activation of dendritic cells (DCs) and promoting the generation of Ag-specific CTLs (7–9). Imiquimod has been shown to be a potent adjuvant for cancer vaccine (3, 8, 10) and to other local treatment, such as cryosurgery (11) and intralymphatic IL-2 (12). Although imiquimod has demonstrated efficacy in superficial cancers, its activity in invasive cancer is less well studied. We evaluated a transgenic model of invasive breast cancer, treating advanced spontaneous tumors arising in the mice, to determine the potential clinical utility and mechanisms of action of TLR-7 ligation in a locally advanced disease setting.

Materials and Methods

Animals

A colony of neu-transgenic mice (strain name, FVB/N-TgN [MMTV-neu]-202Mul) was established in our animal facilities from breeding pairs obtained from the Jackson Laboratory (Bar Harbor, ME). Mice were maintained under strict inbreeding conditions. All of the procedures described in this study were performed in compliance with the University of Washington Institutional Animal Care and Use Committee guidelines. The mice harbor nonmutated, nonactivated rat neu under the control of the mouse mammary tumor virus (MMTV) promoter. Expression of neu under the MMTV promoter results in amplified expression of the protein in the breast epithelium (13). Spontaneous neu mediated breast cancers developed in the animals between 25 and 40 wk old.

Reagents

Five percent imiquimod (Aldara) and a vehicle control cream were supplied by 3M Pharmaceuticals (St. Paul, Minnesota). FBS was obtained from Gemini Bioproducts (Woodland, CA). RPMI 1640, PBS, penicillin-streptomycin, and l-glutamine were obtained from Life Technologies-BRL (Grand Island, NY). Fluorochrome-conjugated Abs targeting CD3, CD4, and CD8 were obtained from BD Pharmingen and eBiosciences (San Diego, CA). The primer and probes for real-time PCR and Taqman Universal PCR Master Mix were obtained from Applied Biosystems (Foster City, CA). The H-2Dβ/RNEU420–429 (PDSDLRDLSVG) tetramer was
obtained from the National Institute of Allergy and Infectious Diseases.

Mice that developed palpable spontaneous mammary tumors were randomly assigned to be treated with imiquimod or a control cream. A thin film (25 μL) of either 5% imiquimod cream or control cream was applied daily to shaved skin over the tumor site on three consecutive days followed by 4 d rest, which was defined as one cycle. The treatment duration was six cycles (6 wk). Tumor size was measured twice per week using Vernier calipers and calculated as the product of length × width × height × 0.5236. In vivo database was presented as the mean ± SEM of individual tumors. Lung metastases were evaluated by a pathologist using fixed tissue slides under microscopic examination. A shorter treatment period, one or two cycles, was also used to evaluate molecular and cellular changes in tumor, spleen, and tumor draining lymph nodes (TDLNs). In some experiments, mice were depleted of specific lymphocytes using mAbs (clone GK1.5 for CD4+ T cells, clone 2.43 for CD8+ T cells, and clone PK136 for NK cells). The mAb (100 μg for CD8+ T cells and NK cells, 300 μg for CD4+ T cells) was given three times during the week before imiquimod treatment and twice per week during the treatment. The depletion efficiency (>95%) was monitored by flow cytometry. For the IL-10 and TGF-β blocking experiments, mice received i.p. injection of 100 μg anti-murine IL-10 mAb (clone JES052A5; R&D Systems, Minneapolis, MN) or anti-murine TGF-β (clone 1D11; R&D Systems) (14) twice per week during imiquimod treatment.

Flow cytometry

The staining of splenocytes was performed as we have previously described (15). Splenocytes were stained with CD3-FITC/RNEU420 tetramer-PE/CD8-PerCP/CD4-APCs. Cells derived from TDLNs were stained with CD80-FITC/CD66-PE/CD11c-PC5 and CD3-FITC/tetramer-PE/CD8-PerCP/CD4-APCs. Tumor infiltrating lymphocytes (TILs) were isolated as previously described (16). All staining was performed at 4°C in the dark for 30 min. To evaluate rat neu and MHC class I (MHC I) and MHC class II (MHC II) expression, tumor cell suspensions were stained using purified anti-neu Ab (Ab-4; Calbiochem, San Diego, CA), biotinylated H2-D0, or biotinylated H1-A0 (BD Pharmingen), followed by secondary staining using goat anti-mouse FITC or streptavidin-PE. For intracellular cytokine analysis, cells were activated with the leukocyte activation kit (BD Biosciences) for 4 h. Next, the cells were first stained with Abs for surface markers (anti-CD3-Alexa488 and anti-CD4-PerCP). After fixation and permeabilization, the cells were stained with anti-IL-10–APC and anti–IFN-γ–PE. For staining of T regulatory (Treg) cells, cells were first stained with anti-CD3-PerCP/anti–CD4-APC/anti–CD25-PE and then stained with anti–Foxp3-Alexa488 after fixation and permeabilization. In some experiments, cells were stained with anti–Foxp3 and anti–IL-10 Abs simultaneously. Data acquisition was done on a FACS Canto flow cytometer (BD Biosciences). List mode data were analyzed using the FlowJo software (Tree Star, OR).

Microarray analysis

Animals were treated with imiquimod for 1 cycle. Two hours after the final treatment, tumors were harvested and immediately put in RNAlater (Ambion, Austin, TX). RNA was extracted using an RNAqueous4PCR kit (Ambion) from four imiquimod-treated tumors (each from an individual mouse) and four control-untreated tumors harvested at the same time. The quality of RNA was assessed for each sample using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). cRNA preparation, probe labeling, and chip hybridization was performed as previously described (17). Labeled cRNA was hybridized to the Sentrix MouseRef-8 Expression BeadChip v1.0a (Illumina, San Diego, CA). Data analysis was performed as previously described (17). Raw data have been deposited in a public database (www.ncbi.nlm.nih.gov/geo/) with the accession number GSE20032.

We used gene set enrichment analysis (GSEA) (18) for pathway analysis. The algorithm was implemented using the Babelomics Web site (19) via KEGG mouse pathway gene sets (www.genome.jp/kegg). Only probes with EntrezGene identifiers were used. In cases in which more than one probe mapped to a single EntrezGene identifier, the one with the highest median signal was used. An enrichment score was calculated for each pathway and normalized for the size of the gene set. Permutation analysis within gene sets was used to determine p values. A false discovery rate q value was generated by comparing the enrichment scores for individual gene sets against the permuted values and against the enrichment scores of all the gene sets. Pathways with p < 0.05 and a false discovery rate q < 0.25 were considered significant (20).

Real time RT-PCR

Tumors from imiquimod-treated tumors (one or two cycles) or control untreated tumors were used for RNA isolation using the RNAqueous4PCR kit. The integrity of RNA was tested using an Agilent BioAnalyzer. cDNA synthesis and real-time PCR was performed as previously described (16).

ELISA for serum IL-10

Serum samples were collected before treatment and at different time points during the treatment via retro-orbital bleeding. The serum samples were frozen until time of analysis. All the samples were measured simultaneously using a murine IL-10 ELISA kit (eBiosciences).

Statistical analysis

Statistical analysis was performed using GraphPad (GraphPad Software, San Diego, CA). Data were analyzed using the Student t test, single factor ANOVA, or two-factor ANOVA. A value of p < 0.05 was considered statistically significant.

Results

CD8+ T cells, induced by TLR-7 ligation, significantly inhibit the growth of spontaneous breast cancers in neu transgenic mice

Topical imiquimod or a control cream was applied at the onset of palpable spontaneous tumors (average tumor size = 93 mm3). As shown in Fig. 1A, tumor growth was significantly inhibited in the group of mice that received imiquimod. After two cycles of therapy (day 15), imiquimod-treated tumors were significantly smaller than vehicle-treated tumors (125.3 ± 27.6 mm3 versus 302.8 ± 62.5 mm3; p = 0.03). After six treatment cycles, the average tumor size was 73 ± 5 mm3 in the imiquimod group versus 1366 ± 262 mm3 in controls (p = 0.01; Fig. 1A). At the end of 6 wk of treatment, we evaluated the animals for the presence of spontaneous lung metastasis that is well described in these animals (13). Lung metastasis was observed in 30% of controls (n = 10; Fig. 1B) but in none of the imiquimod-treated mice (n = 21; p = 0.03), suggesting that imiquimod has a systemic antitumor effect in addition to directly inhibiting tumor growth.

Evaluation of TILs showed a significant increase in the ratio of CD8+/CD4+ T cells in imiquimod-treated mice (0.78 ± 0.17 in control tumors versus 2.62 ± 0.54 imiquimod-treated mice; p = 0.007; Fig. 1C). Tumor cells derived from imiquimod-treated mice expressed higher levels of MHC I (mean fluorescence intensity, 88.5 ± 0.5 in control versus 237.0 ± 4.0 in treated tumors; p = 0.01; Figs. 1D, 1E). The expression of MHC II was not changed by the treatment (p = 0.21). To examine whether the observed MHC I upregulation on the surface of tumor cells was a direct effect of imiquimod, we treated MMC—a tumor cell line derived from syngeneic tumors in these mice—with imiquimod. Results demonstrate that there was no upregulation of MHC I after direct stimulation (p = 0.37; imiquimod versus PBS-treated cells; Supplemental Fig. 1), indicating that the effect on MHC I after in vivo treatment was probably indirect via induction of proinflammatory cytokines. Furthermore, we found that these tumor cells did not express TLR-7 (Supplemental Fig. 2).

Evaluation of rat neu Ag-specific CD8+ T cells among splenocytes using tetramer staining demonstrated a significant increase in imiquimod-treated mice (0.99 ± 0.1% in control versus 3.3 ± 0.7% after 1 wk imiquimod treatment; p = 0.007), which persisted throughout treatment (Fig. 1E). To further determine the role of CD8+ T cells in mediating the antitumor effect of imiquimod, mice were selectively depleted of CD4+, CD8+ T cells, or NK cells during treatment. As shown in Fig. 1F, the antitumor effect of TLR-7 ligation was completely abrogated by CD8+ T cell depletion.
but not by CD4+ T cell or NK cell depletion. Histologic examination of the tumors after 6 wk of treatment showed hemorrhage and fibrin deposition as well as cholesterol clefts, suggesting excessive inflammation induced by the imiquimod treatment (Fig. 1G).

Once imiquimod treatment was stopped, however, tumor growth progressed again. Fig. 1H shows the regrowth of tumors in mice after 3 wk imiquimod treatment (Fig. 1H). Tumors grew back similarly even after longer treatment (data not shown). To determine the potential mechanisms of failure of the induced adaptive immune response, we examined serial gene expression changes in the tumors during imiquimod treatment.

**TLR-7 ligation elicited the upregulation of both pro- and anti-inflammatory cytokine genes in the tumor as well as elevated serum levels of IL-10**

Gene expression analysis demonstrated that after one cycle of imiquimod treatment, there was significant change in the expression of 646 genes among the total of 22,000 examined genes ($p < 0.05$). GSEA exploring all KEGG mouse pathways showed that multiple immune response pathways were activated in the tumor, including TLR signaling pathways and cytokine-cytokine receptor pathways (Supplemental Table I). Both proinflammatory (e.g., IL-2, IFN-γ, TNF) as well as anti-inflammatory (e.g., IL-10) genes were upregulated in the cytokine receptor pathway after imiquimod treatment (Fig. 2A). Validation of expression of TNF-α, IFN-γ, TGF-β, and IL-10 (Fig. 2B–E) was performed by quantitative PCR. Although the levels of TNF-α and IFN-γ increased to 4.4 fold and 8.0 fold of control after 1 wk imiquimod treatment ($p = 0.03$ and 0.02, respectively; Fig. 2B, C), the level of IL-10 was increased to 42.6-fold of control ($p = 0.006$; Fig. 2D). This increase was maintained at 2 wk of treatment. Of note, the level of TGF-β gene expression in the tumor was not changed by imiquimod treatment; the expression level at week 1 was 0.74-fold of control ($p = 0.24$; Fig. 2E). We also examined the expression level of IL-13, another immunosuppressive
FIGURE 2. TLR-7 ligation elicited the upregulation of both proinflammatory and anti-inflammatory cytokine genes in the tumor as well as elevated serum levels of IL-10. A, Heat map of genes in the cytokine-cytokine receptor pathway, which was shown to be upregulated by GSEA. Red indicates high expression level, and blue indicates low expression level. The four columns on the left are tumor samples from the control group, and the four columns on the right are tumor samples from imiquimod-treated mice. All genes are normalized to β-actin. *p < 0.05, from control. B–E, The relative expression levels (mean ± SEM) of TNF-α (B), IFN-γ (C), IL-10 (D), and TGF-β (E) in control untreated tumors and tumors from 1 wk or 2 wk imiquimod-treated mice. All genes are normalized to β-actin. *p < 0.05, from control. F, The level of serum IL-10 from control (○) or imiquimod-treated (●) mice before treatment or at 1, 2, 3, 4, and 5 wk after treatment. Each data point represents the value from an individual mouse. The horizontal bar represents the mean in each group. The experiment was repeated twice with similar results.

cytokine that has been indicated to play a role in tumor escape (21, 22). Although there was a trend of increase in imiquimod-treated tumors, it was not statistically significant, and the level of induction (<2-fold) was minimal compared with IL-10 (Supplemental Fig. 3).

We questioned whether the observed gene expression changes resulted in systemic levels of cytokines that could impact T cell function. We collected serial blood draws from mice before and at various time points during imiquimod treatment. As shown in Fig. 2F, serum IL-10 levels became significantly elevated in the imiquimod-treated animals (p = 0.01). Serum IL-10 levels seem to fluctuate in imiquimod-treated mice and not all the mice had elevated IL-10 levels at the same time, but all of them had elevated IL-10 levels for at least one of the posttreatment time points. Serum levels of TNF-α or IFN-γ were not detectable in either the imiquimod or control group at all the time points.

Both CD4+ Foxp3+ Treg and IL-10 secreting regulatory Th1 cells are induced by TLR-7 ligation

It is known that CD4+CD25+Foxp3+ Treg cells can exert their inhibitory effect partially via secretion of IL-10 (23), so we examined whether Foxp3+ expressing T cells were the source of serum IL-10. As shown in Fig. 3, imiquimod treatment resulted in elevated levels of CD4+CD25+Foxp3+ Treg cells in spleen (6.19 ± 0.33% versus 10.70 ± 0.76%; p = 0.0006; Fig. 3A), TDLNs (4.41 ± 0.32% versus 7.48 ± 0.72%; p = 0.0046; Fig. 3B), and TILs (1.55 ± 0.41% versus 3.01 ± 0.25%; p = 0.026; Fig. 3C). The ratio of CD8+ T cells to Foxp3+ Treg cells decreased after imiquimod treatment in spleen (3.04 ± 0.14 to 1.78 ± 0.28; p = 0.004), TDLNs (4.48 ± 0.40 to 2.96 ± 0.21; p = 0.010), and TILs (12.35 ± 2.73 to 4.38 ± 0.55; p = 0.038). However, simultaneous staining of IL-10 and Foxp3 showed that IL-10 is secreted mainly from Foxp3-negative T cells, indicating that Foxp3+ Treg cells are not the major source of elevated serum IL-10 (Fig. 3D, 3E).
Intracellular cytokine staining using splenocytes from control and imiquimod-treated mice demonstrated that imiquimod treatment increased T cells secreting IFN-γ (3.45 ± 0.37% versus 9.49 ± 0.93%; \( p = 0.0003 \); Fig. 4A) and IL-10 (0.30 ± 0.05% versus 1.79 ± 0.64%; \( p = 0.04 \); Fig. 4B). Furthermore, imiquimod induced a population of CD4+ T cells secreting both cytokines (Fig. 4C, 4D). The dual cytokine-secreting cells, which are rarely found in T cells from control tumor-bearing mice (0.11 ± 0.01%), increased more than 10-fold to 1.53 ± 0.52% in splenocytes derived from imiquimod-treated mice (\( p = 0.02 \), compared with control).

**FIGURE 3.** CD4+CD25+Foxp3+ Treg cells are induced by TLR-7 ligation. Representative flow graphs and summary graphs showing CD25+Foxp3+ cells in spleens (A), TDLNs (B), and TILs (C) from control and imiquimod-treated (6 wk) mice. In the flow graphs, the cells shown were first gated as CD3+ CD4+ T cells. In the summary graph, each data point represents the value from an individual mouse. The horizontal bar represents the mean in each group (\( A–C \)).

D and E, IL-10 is secreted mainly by Foxp3+CD4+ T cells. D, A representative flow graph of splenocytes from imiquimod-treated mice costained with Foxp3 and IL-10. The cells shown are first gated as CD4+ T cells. E, A summary graph of the percentages of IL-10 secreting CD4+ T cells (among total CD4+ T cells) that are Foxp3+ or Foxp3−. The column represents mean ± SEM in three imiquimod-treated mice. \(*p < 0.01. **Similar results were obtained from two independent experiments.

IL-10 blockade augments the antitumor effect of imiquimod and results in long-term survival of imiquimod-treated mice

To determine whether imiquimod-induced IL-10 secretion by T cells was operative in treatment failure, we treated mice with an anti–IL-10 mAb. An anti–TGF-β mAb was also evaluated to counteract TGF-β secretion by the elevated levels of Treg cells. As shown in Fig. 5, the control untreated mice died at 32 ± 8 d after the start of the experiment. Imiquimod alone (6 wk treatment) enhanced the survival time to 54 ± 5 d (\( p = 0.0004 \) from control). Blockade of TGF-β did not prolong the survival time (52 ± 3 d; \( p = 0.26 \) from imiquimod alone). Blockade of IL-10 significantly enhanced the
antitumor effect of imiquimod and significantly prolonged survival ($p = 0.002$, from imiquimod alone).

**Discussion**

Imiquimod is commonly used in the treatment of genital warts and superficial basal cell carcinomas as well as other noninvasive cancers (24, 25). When topical imiquimod was evaluated as a treatment for invasive melanoma, however, sustained remissions were rarely observed. Recent evidence suggests that only metastatic lesions located in the dermis will respond to the topical therapy (26). Using a mouse model of invasive breast cancer, we have found that TLR-7 ligation by imiquimod results in acute tissue inflammation and significant inhibition of spontaneous mammary tumor growth in neu-transgenic mice. However, tumors grew back after treatment withdrawal. These studies demonstrate that imiquimod therapy not only stimulates proinflammatory cytokines and augments Ag-specific CD8+ T cells, but also stimulates the proliferation of Foxp3+ regulatory T cells, IL-10 secreting CD4+ T cells, and dual secreting IFN-γ+IL-10+CD4+ T cells, all of which have been shown to be involved in self regulation of inflammation. IL-10, but not TGF-β blockade, significantly enhanced the antitumor effect of imiquimod, resulting in markedly prolonged survival of treated mice.

The observation that acute inflammation can lead to tumor destruction has been previously reported for a variety of local treatments, such as intratumoral bacterial/viral injection, cytokine injection, and cryoablation (27–29). All of these treatments have been shown to induce intratumor inflammation that favors cross-priming of tumor Ags and upregulation of MHC I. The activation of TLR might be the common underlying mechanism for many of these local treatments. Our study demonstrated that imiquimod not only induces local inflammation, but also has a systemic effect, as evidenced by the absence of lung metastasis in treated mice and the systemic increase in Ag-specific CD8 T cells, which seems to mediate the antitumor effect of imiquimod. This finding is consistent with previous reports that topical imiquimod as a vaccine adjuvant can augment CTL response to protein, peptide, or DC vaccine (4, 8, 10, 30). The potential mechanism that can account for the failure of TLR agonists in eradicating cancer, however, is not well defined. Our study demonstrated that imiquimod can activate the self-regulatory feedback, including Treg cells and IFN-γ/IL-10–secreting CD4 T cells, and blockade of IL-10 can augment the antitumor effect of imiquimod.

The interplay of TLR ligation with DC activation and Treg cell stimulation is complex. Both DCs and Treg cells have been shown to express functional TLRs, and activation via the receptor can have an immune-stimulating or -suppressing effect (31). Using RT-PCR, we found that the expression of TLR-7 on Treg cells (CD4+CD25+) is significantly lower than that on DCs, but higher than that on CD4+CD8+ T cells (data not shown). Whether this relatively higher expression of TLR-7 on Treg cells is related to the observed imiquimod-induced increase in Treg...
cells, both systemically and in the tumor, remains to be investigated. Previous studies have reported contradicting effects of imiquimod on Treg cells (32, 33). In a study on the use of imiquimod to treat squamous cell carcinomas of the skin in immunosuppressed organ transplant patients in whom Treg are abundant (32), investigators demonstrated that imiquimod treatment reduced the numbers of Foxp3-expressing Treg cells in treated lesions. Moreover, evaluation of the infiltrating Treg cell population suggested that TLR-7 ligation resulted in decreased expression of Foxp3, IL-10, and TGF-β in those cells (32). A more recent publication by Forward et al. (33) showed that TLR-7 agonists including imiquimod enhance the immunosuppressive activity of murine CD4+CD25+ Treg cells. Whether the controversial data are due to different host system, mouse versus human, or different treatment method, remains to be investigated in future studies.

Although Treg cells have been shown to secrete IL-10 (23, 34), we found that the Treg cells induced by imiquimod expressed little IL-10, leading us to assume that the CD4+CD25+Foxp3+ population did not substantially contribute to elevated serum IL-10 levels observed in treated animals. Intracellular cytokine staining analysis did not substantially contribute to elevated serum IL-10 levels observed in treated animals. Th1 can also become self-regulatory by cosecreting IFN-γ (41). Ag-experienced T cells appear to require IFN-γ for optimal function, leading to chronic infection (42). Serum containing soluble IL-10 results in upregulation of IL-10, inhibiting effector T cell function and restoring the Th1/Th2 balance in patients with metastatic melanoma. Br. J. Dermatol. 159: 606–614.


Our work suggests that a mechanism of treatment failure with TLR-7 ligation is the host self-regulatory response to induced inflammation. Moreover, IL-10 secretion appears to be an important mediator of immunosuppression, and IL-10 blockade greatly enhanced the antitumor effect of TLR-7 ligation. Topical use of imiquimod in breast cancer with inflammatory breast cancer or cutaneous metastasis of breast cancer is currently tested in a clinical trial in our group. Whether IL-10 blockade is feasible in patients with cancer needs to be further investigated, because this study did not address the potential of developing immune-related side effects. A recent publication on patients with cancer receiving anti–CTLA-4 therapy reported that decreased levels of IL-10 coincided with the patient’s immune-related adverse events (47). This finding indicates that caution needs to be taken before the IL-10 blockade approach can be translated into cancer treatment.

Disclosures

The authors have no financial conflicts of interest.

References


