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Systemic cancer immunotherapy with Toll-like receptor 7 agonists

Timing is everything

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Toll-like receptor (TLR) 7 agonists represent a promising strategy for the immunotherapy of cancer. We have recently investigated the influence of TLR tolerance on the efficacy of systemic tumor treatment with TLR7 ligands. We propose that considering the kinetics of receptor sensitivity highly improves the outcome of cancer immunotherapy.

Toll-like receptors (TLRs), which sense conserved molecular patterns from microbial pathogens, trigger a cascade of events characterized by the secretion of proinflammatory cytokines that results in the stimulation of innate and adaptive immunity. Agonists of TLRs are therefore of major interest for the immunotherapy of cancer. Imiquimod, a small molecule agonist of TLR7, is already successfully used for the topical treatment of skin neoplasias such as basal cell carcinoma.[1] In view of the potential use of TLR7 ligands for the treatment of non-skin cancer, we and others have investigated the ability of TLR7 agonists to induce systemic immune responses. A combined schematic view of the anti-tumor activity of TLR7 agonists as demonstrated by our recent work is depicted in **Figure 1**. We have shown that the systemic application of TLR7 ligands functionally activates both $CD8^+$ T cells and NK cells, two major effector cell types for anticancer responses.[2] Furthermore, TLR7 activation blocks the suppressive function of regulatory T cells that contribute to cancer-associated immune suppression.[3] Finally, we have shown that IFN-α produced by plasmacytoid dendritic cells upon TLR activation reduces the immunosuppressive activity of myeloid-derived suppressor cells (MDSC), a cell population that accumulates in cancer patients and suppresses T cell responses.[4] We have elucidated the combined molecular basis for these effects by demonstrating that they are mediated by proinflammatory cytokines, in particular IL-6, and type I interferon secreted by dendritic cells following their activation through TLR7.[2-4] Thus, TLR7 agonists appear to fulfill many of the requirements for an effective systemic immunotherapy.

The few studies investigating systemic application of agonists for TLR7 or its close homolog TLR8 in cancer patients have however shown little efficacy.[5,6] Treatment in these studies consisted of two to three weekly applications. Given the fact that cytokine secretion is crucial for the therapeutic effect of TLR7,[7] we speculated that systemic cancer therapy with immune response modifiers might be limited by a phenomenon called TLR tolerance. TLR tolerance is characterized by blunted cytokine secretion after repetitive receptor stimulation and has been described for several TLRs, including TLR7.[8] Indeed, we recently demonstrated for the first time that TLR tolerance influences the efficacy of TLR stimulation for the immunotherapy of cancer.[9] In this study we analyzed the kinetics of repeated TLR7 stimulation with the small molecule agonist R848 in mice. We showed that a single injection of R848 blocks the cytokine response to a second stimulation for a time frame beginning 48 h after the first injection and lasting for up to five days. In contrast, repeated stimulation within the first 24 h resulted in an enhanced response. These findings suggested that injections every second to third day, as performed in clinical studies of TLR7 agonists, would maintain TLR7 in a refractory state. We designed a protocol of fractionated cancer therapy with R848 in cycles separated by five day intervals to take advantage of the initial receptor priming and to avoid tolerance. This protocol blocked tumor growth in a murine cancer model with a higher efficiency than the schedule used in clinical studies, although the cumulated dose was lower.[9]

We observed TLR7 tolerance in myeloid dendritic cells, in which the secretion of proinflammatory cytokines was inhibited, but also in plasmacytoid dendritic cells, where type I interferon secretion was suppressed.[9] We showed in vivo that tolerance correlated well with decreasing levels of IRAK-1, an essential adaptor molecule for type I interferon and cytokine production. Furthermore, tolerance was not restricted to TLR7: we demonstrated the occurrence of heterotolerance, since TLR7 stimulation led

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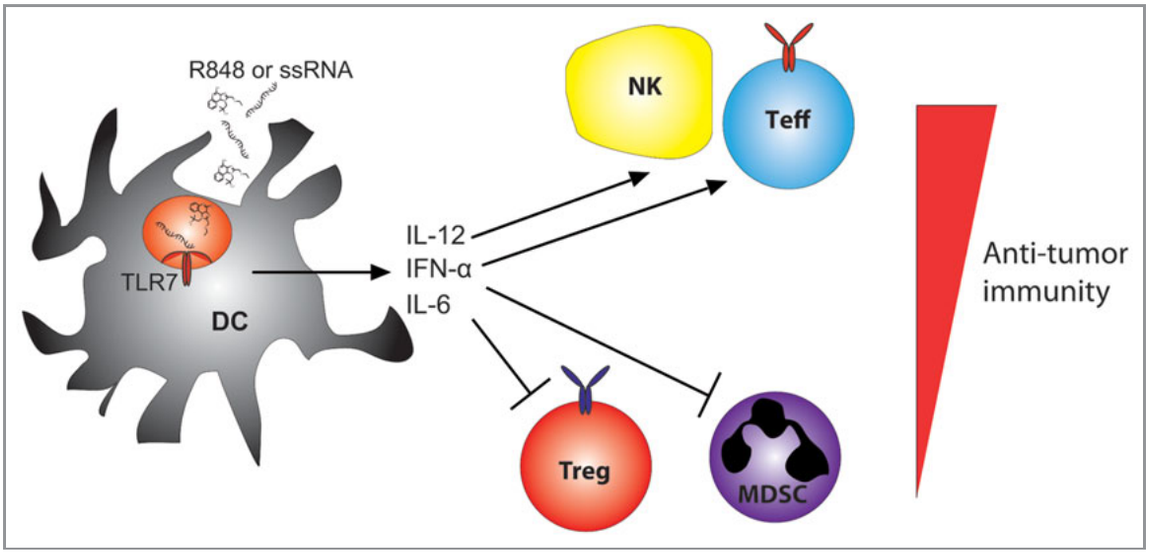

Figure 1. Overview of the cellular mechanisms of TLR7-targeting therapy. DC: dendritic cell, Teff: effector T cells, Treg: regulatory T cell, MDSC: myeloid-derived suppressor cell, NK: natural killer cell, ssRNA: single-stranded RNA.

to blunted cytokine secretion by subsequent TLR2, 4 and 9 stimulation. Given the global occurrence of tolerance, our results may impact all therapeutic approaches relying on repetitive stimulation with immune response modifiers.

Interestingly, in contrast to the secretion block observed for proinflammatory cytokines, secretion of the suppressive cytokine IL-10 was enhanced in tolerized dendritic cells. Although not causally involved in the induction of TLR7 tolerance,[9] IL-10 may also limit the efficiency of immunotherapy. It was recently demonstrated that IL-10 suppresses proinflammatory cytokine secretion following simultaneous stimulation of two independent TLR pathways, thus reducing the number of tumor-specific CD8+ T cells and impairing the efficiency of cancer immunotherapy.[10] It is probable that during simultaneous stimulation of two independent TLR pathways, both are affected by tolerance induction concomitantly. The application of a fractionated schedule to prevent tolerance in protocols employing combinatorial stimulation may therefore prove beneficial by both enhancing the secretion of proinflammatory cytokines and preventing high production of IL-10. In conclusion, we suggest that a careful evaluation of the timing of TLR stimulation and the profile of cytokines induced is essential for effective systemic immunotherapy with TLR agonists.

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