Toll-like Receptors in Tumor Immunotherapy

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Abstract

Lymphodepletion with chemotherapeutic agents or total body irradiation (TBI) before adoptive transfer of tumor-specific T cells is a critical advancement in the treatment of patients with melanoma. More than 50% of patients that are refractory to other treatments experience an objective or curative response with this approach. Emerging data indicate that the key mechanisms underlying how TBI augments the functions of adoptively transferred T cells include (*a*) the depletion of regulatory T cells (T_{reg}) and myeloid-derived suppressor cells that limit the function and proliferation of adoptively transferred cells; (*b*) the removal of immune cells that act as "sinks" for homeostatic cytokines, whose levels increase after lymphodepletion; and (*c*) the activation of the innate immune system via Toll-like receptor 4 signaling, which is engaged by microbial lipopolysaccharide that translocated across the radiation-injured gut. Here, we review these mechanisms and focus on the effect of Toll-like receptor agonists in adoptive immunotherapy. We also discuss alternate regimens to chemotherapy or TBI, which might be used to safely treat patients with advanced disease and promote tumor regression.

> *It is the information carried by the bacteria that we cannot abide. The gram-negative bacteria are the best examples of this. They display lipopolysaccharide endotoxin in their walls, and these macromolecules are read by our tissues as the very worst of bad news. When we sense lipopolysaccharide, we are likely to turn on every defense at our disposal*. . . Lewis Thomas, *New England Journal of Medicine,* 1971

Historical Perspective on Exploiting Bacteria to Treat Cancer

Over a century ago, William B. Coley observed spontaneous tumor regression in some of his patients with bacterial infection (1). He hypothesized that the tumor regression was caused by infection and designed a mixture of bacteria consisting of heat-killed cultures of *Streptococci* and *Serratia marcescens* known as Coley's toxins. He reported success using these mixtures in patients with soft tissue sarcoma. Subsequent investigators, however, failed to reproduce these findings (2). Nonetheless, Coley's notion that bacteria can activate immune responses to cancer cells persists to the present day.

Bacterial mixtures as activators of immune responses could be understood in the danger model formulated by Polly Matzinger in the early 1990s (3,4). According to this model of immune activation versus tolerance, tumor antigens would be viewed as dangerous to the immune system in the presence of bacteria. A molecular understanding of how host-derived bacteria stimulate the immune system was later elucidated by Charles Janeway and colleagues (5–7) who found that bacteria contain conserved pathogen-associated molecular patterns such as

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lipopolysaccharide (LPS) and bacterial DNA that can ligate pattern recognition receptors, such as the Toll-like receptors (TLR) of the innate immune system (8–11). Engagement of TLRs on antigen presenting cells (APC; such as dendritic cells) of the innate immune system results in their maturation and migration to lymph nodes where they initiate adaptive immune responses (12,13).

TLRs in Intestinal Immune Health and Disease

It is now clear that recognition of host-derived bacteria by TLRs is vital for intestinal health, exerting a variety of structural, metabolic, and protective effects on the intestinal mucosal barrier (14–17). Medzhitov and colleagues first found that TLR detection of gut microbes could protect mice from life-threatening colitis. They found that mice genetically deficient in My D88 (a molecule important for TLR recognition of gut microbes) succumbed to increased disease pathogenesis as evidenced by pronounced gut injury and related mortality compared with wild-type mice (18). Conversely, gut injury and mortality was increased in wild-type mice with colitis upon removal of gut microbes using broad-spectrum antibiotics. Interestingly, administrating LPS to their drinking water rescued antibiotic-treated wild-type mice from disease pathogenesis, indicating that TLR recognition of microbial products was surprisingly important for maintaining intestinal immune health (18).

TLR recognition of gut microflora can also be deleterious to the host, especially if the host is rendered lymphopenic by a chronic infection or by lymphodepleting preparative regimens (19–21). Translocated colonic microbes have been implicated in exacerbating graft-versus-host disease in patients receiving allogeneic stem cell transplant (22–27) and have recently been reported to cause systemic immune activation in patients with chronic HIV infection (28,29). Thus, it is clear that commensal gut microflora can exacerbate unwanted immune responses, but the potential for exploitation in T cell–based antitumor immunotherapies remains to be fully explored.

Recent Efforts in Exploiting TLRs for the Treatment of Cancer

Given the notion that bacteria might increase antitumor immunity, the question arises "Can bacterial TLR agonists augment cancer immunotherapy?" Early studies indicated that inserting model tumor-associated antigens into viruses, which contain TLR agonists, can augment their immunogenicity and function as tumor vaccines (30–32), but these studies largely used very early treatment models in which tumors were not yet vascularized. In humans, cancer vaccines have not proven to be consistently therapeutic even when used under circumstances in which recombinant and synthetic vaccines are capable of activating the innate immune system (33).

Recently, Speiser and colleagues have made efforts to use TLR agonists in conjunction with vaccination in patients with melanoma (34,35). They found that combining TLR9 agonist CpG ODN 7909 (a 24-mer oligodeoxynucleotide containing 3CpG motifs) with a Melan $A_{26–35}$/ MART1 peptide and incomplete Freund's adjuvant increased the number of MART1-specific T cells by >10-fold compared with vaccination without CpG. This heightened immune response, however, did not promote tumor regression (35). These findings might imply that the MART1 T cells induced by CpG ODN 7909 and vaccination are functionally tolerized. The tumor-reactive T cells might be tolerized by regulatory T cells (T_{reg}) cells, as hypothesized by the same investigators, who reported that T_{reg} cells were elevated in the tumors of vaccinated patient's receiving CpG ODN 7909 (36).

Adoptive Immunotherapy

Adoptive transfer of autologous tumor-specific T cells, as initially reported in 1988, has been shown to reproducibly mediate the destruction of bulky tumors in ~30% of the patients treated

(37,38). This approach involves growing tumor-infiltrating lymphocytes *ex vivo* from the resected tumor nodules of patients and then adoptively transferring them into the patient in conjunction with bolus high-dose interleukin-2 (IL-2; refs. 37,38). Although this approach is cumbersome, it has a number of advantages. One can administer large numbers of naturally occurring or genetically engineered cells with high avidity for tumor antigens (39,40). Both CD4^{+} and CD8^{+} T cells have been shown to be capable of recognizing tumor antigens and to potentially play a role in the antitumor immune response (41–45). These cells can be selected for particular functions (46), they can be activated from their poorly functional state *in vivo* (47), and they can be programmed for optimal function (48). After adoptive transfer into a tumor-bearing host, they are capable of massive expansion in both mice and humans (49,50). Adoptively transferred T cells are capable of trafficking to virtually every somatic site (51). Despite all of these advantages, the adoptive transfer of cells triggered objective immune responses in only a small minority of patients until investigators began to use lymphodepleting preparative regimens to provide an altered environment for transferred cells (49,52–56).

Lymphodepletion Augments Adoptive Immunotherapy

Adoptive transfer of *ex vivo* expanded antitumor T cells after a nonmyeloablative lymphodepleting preparative regimen with chemotherapeutic reagents is the most effective treatment for patients with metastatic melanoma. When antitumor T cells are given after lymphodepletion, ~50% of patients refractory to other treatments will experience objective clinical responses with this treatment (Fig. 1A and B; refs. 49, 55, 57). Not all patients' tumors yield antitumor T cells, however, and only about a third of the patients experience durable tumor regressions. Thus, there are significant efforts to continue to improve the current therapies. Preclinical data show that increasing the intensity of lymphodepletion to a myeloablative preparative regimen that requires hematopoietic stem cell (HSC) transplant might further improve the effectiveness of adoptive cell transfer. This approach is currently being examined in clinical trials at the NIH, National Cancer Institute, Surgery Branch (58–60). Preliminary findings indicate long-term responses in patients pretreated with chemotherapeutics agents in conjunction with 200 cGy total body irradiation (TBI; Fig. 1C) or 1,200 cGy TBI (six fractionated doses of 200 cGy TBI, twice a day for 3 days) plus HSC transplant (Fig. 1D).

The concept that lymphopenia-induced homeostatic expansion of T cells intensifies immune responses to tumor antigens and facilitates potent antitumor immune responses was first reported by Fefer and colleagues in the late 1960s (61). They found that i.p. administration of lymphocytes in conjunction with chemotherapy could treat mice bearing virally induced lymphomas (61). Likewise, other groups in the early 1980s found that this approach could mediate the regression of established sarcomas in mice and rats (62,63). More recently, Dummer and colleagues found that homeostatic expansion of autologous CD8^{+} T cells can inhibit tumor growth. Importantly, CD8^{+} T cells from these mice mediated tumor-specific cytotoxicity and IFN-γ production associated with long-term tumor-specific memory (64). In addition, tumor growth was profoundly inhibited in irradiated animals vaccinated with tumor lysate–pulsed dendritic cells (65). This work clearly showed that lymphodepletion can enhance T cell–mediated antitumor immune responses.

We have explored the use of lymphodepletion with a nonmyeloablative preparative conditioning regimen using 5 Gy TBI in a model of adoptive immunotherapy using CD8^{+} pmel-1 T cells that are specific for the self/tumor antigen gp100 (66–68). The administration of 5 Gy TBI before a tripartite regimen consisting of adoptive transfer of transgenic pmel-1 cells, vaccination encoding gp100, and IL-2 significantly enhanced the destruction of large, established B16 tumors (69). The mechanisms underlying this improvement are multifold and have been substantially elucidated using varieties of knockout and transgenic animals (Fig. 2).

These mechanisms include the removal of inhibitory lymphocytes such as regulatory T cell and cytokines sinks, elevation of homeostatic cytokines, and activation of the innate immune system (Fig. 3).

Mechanisms Underlying the Effectiveness of TBI in Adoptive Immunotherapy

Depletion of T_{reg} cells

Lymphodepletion before the adoptive transfer of T cells can also reduce the numbers of $CD4^+$ $CD25^+FoxP3^+$ T_{reg} cells whose generation and function are reviewed elsewhere (70, 71). T_{reg} cells maintain tolerance to self-antigens by blocking the functionality of effector T cells (72–80), as reported by North and colleagues more than two decades ago (81). Emerging data indicate that the CD39 and CD73 are surface markers for T_{reg} cells that impart a specific biochemical signature characterized by adenosine generation (82). These phenotypic markers might have more functional relevance than the cell surface marker CD25 for cellular immunoregulation. It is now clear that removal of T_{reg} cells by 5 Gy TBI improve the function of adoptively transferred cells (Fig. 3; refs. 58,78). Another category of immune cells that have been reported to induce lymphocyte dysfunction and promote tumor growth are designated myeloid-derived suppressor cells (MDSC; refs. 83–89). Removal of these cells might also contribute to the effectiveness of TBI.

Removal of cytokine sinks

Lymphodepletion with nonmyeloablative 5 Gy TBI augment the effectiveness of adoptively transferred cells in part by depleting the lymphoid compartment that consumes homeostatic cytokines (cytokine sinks such as host natural killer, $CD4^+$ and $CD8^+$ T cells; refs. 69,78). Removal of this compartment increase the availability of homeostatic γ-chain cytokines such as IL-7, IL-15, and possibly IL-21. An increased availability of these cytokines for the adoptively transferred cells may enhance their function and enable them to destroy bulky nonimmunogenic tumors (58,69).

Activation of innate immunity via translocated microbes

Interestingly, removal of T_{reg} cells and cytokine sinks do not fully account for the dramatically improved tumor regression resulting from TBI. We recently found that $Rag2^{-/-}$ $\gamma_c^{-/-}$ mice, which lack both T_{reg} cells and sinks for homeostatic cytokines, benefited from TBI preconditioning, an unexpected finding given that these mice are deficient in all lymphocyte subsets. These data indicate the existence of another mechanism by which TBI enhances adoptive immunotherapy (90).

TBI has recently been reported to increase antigens on the cell surface of the tumor stroma (91,92). Increased expression of antigens was reported to contribute to elimination of the immunogenic tumors by T cells. We found that local irradiation at the tumor site of up to 10 Gy before adoptive cell transfer, however, had virtually no effect on the growth rate of established B16 melanoma (69). Conversely, TBI with 5 Gy before adoptive cell transfer enhanced tumor destruction even when the tumor was shielded from radiation. This indicated that the observed enhancement of $CD8^+$ T-cell function after TBI did not directly result from its influence on the tumor, but rather resulted from its effect on the host cells. This finding is in accordance with earlier studies by Hellstrom in 1978, who first reported the irradiation might be beneficial for the treatment of MCA-1315 tumors in mice because of the effect of radiation on host but not tumor cells (93).

Additional investigation conducted in our laboratory revealed that radiation-induced injury to the gut permitted translocation of microbes to the mesenteric lymph nodes and systemic liberation of bacterial-derived LPS (90). Bacterial translocation resulted in activation of the

innate immune system, as indicated by an increase in the splenic $CD11c^+$ $CD86^{hi}$ dendritic cells, which are capable of activating adoptively transferred T cells (94–96). In addition, higher levels of pro-inflammatory cytokines IL-1β, IL-6, and tumor necrosis factor-α were found in the serum of irradiated mice (90). Furthermore, IL-12, a well-described enhancer of $CD8^+$ T-cell function (97,98), was found in the serum as well (90).

Neutralization of gut microbes with ciprofloxacin reduced the absolute numbers of activated host dendritic cells and impaired tumor destruction by the adoptively transferred cells in irradiated mice (90). These data have shown that innate activation by the translocated microbes is surprisingly important for improving T cell–based immunotherapy. As cancer immunotherapy develops, it is important to understand the effect of these treatments on host-microbe homeostasis and the role of gut microbes in antitumor immunity. A better understanding of this mechanism might allow us to optimize our therapies to enhance clinical responses.

Translocation of Microbial LPS Augments Adoptive Immunotherapy *via* TLR4 Signaling

Microbes that translocate across the perturbed gut contain a plethora of TLR agonists (i.e., lipoproteins, lipoteichoic acid, peptidoglycan, flagellin, bacterial DNA, and LPS; refs. 11,13, 99). Thus, any of these agonists might have been responsible for enhancing adoptive immunotherapy. We found, however, that LPS is principally responsible for the effectiveness of TBI because neutralizing it with polymyxin B, a cyclic cationic polypeptide antibiotic that blocks the biological effect of Gram-negative LPS (100), also impaired tumor destruction mediated by the adoptively transferred cells.

LPS, which binds to the soluble LPS binding protein, activates innate immunity primarily through engagement of TLR4, which acts in conjunction with accessory CD14 and MD2 molecules (refs. 101,102;Fig. 4). These accessory molecules are important for stabilizing TLR4. TLR4 engagement induces nuclear factor-κB activation via multiple pathways (i.e., MyD88, TRIF, TIRAP, and TRAM pathways; refs. 103,104), leading to the production of pro-inflammatory cytokines, up-regulation of costimulatory molecules, and greater expression of MHC class II on phagocytes (Fig. 4). Innate activation via TLR4 engagement of microbial LPS was important for the effectiveness of TBI because removal of LPS signaling components using mice genetically deficient in CD14 or TLR4 reduced destruction of large tumors by the adoptively transferred cells (90).

Depletion of Sinks and Suppressors: Requirements for TLR Agonists to Enhance Adoptive Immunotherapy

Investigators have previously reported that TLR agonists can inhibit tumor growth in prevention models of adoptive immunotherapy (105–108). Because of this finding and because endogenous microbial LPS plays a role in the effectiveness of TBI, we hypothesized that exogenous administration of LPS to nonirradiated mice might replace preconditioning animals with TBI. We found, however, that exogenous LPS alone does not mediate regression of large, established, nonimmunogenic B16 tumors in nonirradiated mice receiving adoptive immunotherapy in comparison with mice irradiated with 5 Gy TBI. Furthermore, administration of other TLR agonists [i.e., imiquimod, zymosan, poly(I:C), or CpG] alone were likewise ineffective.[1] Thus, in contrast to prevention models, TLR agonist cannot replace the effectiveness of TBI in adoptive cell transfer models with large, established tumors.

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[1]C. Paulos, unpublished data.

What is required for TLR agonists to mediate destruction of large tumors by adoptively transferred cells? The answer to this question is clear from mechanisms underlying the effectiveness of TBI (58,59,69,78,90,109). TBI enhances adoptive immunotherapy via removal of cytokine sinks, depletion of T_{reg} cells, and activation of innate immunity via TLR ligation. In fact, we found that depletion of cytokine sinks alone (with a single administration of natural killer–depleting antibody), removal of T_{reg} cells alone (with a single administration of CD4-depleting antibody), activation of the innate immune system via TLR4 signaling alone (with systemic administration of LPS at 1 day after adoptive cell transfer), or any two combinations of the three mechanisms underlying the effectiveness of TBI induces nominal antitumor immune responses by the adoptively transferred cells in nonirradiated mice with bulky tumors (90). Destruction of bulky tumors achieved in irradiated mice is only achieved in nonirradiated mice by mimicking all three mechanisms underlying the effectiveness of TBI. These findings are important because they define the variables required for TBI to improve adoptive immunotherapy for cancer.

Administration of LPS to Irradiated Animals Enhances Adoptive Immunotherapy

We recently reported that exogenous administration of LPS to irradiated animals further enhanced the proliferation and function of adoptively transferred cells, resulting in long-term cures and enhanced autoimmune vitiligo in these animals. (90). These data indicated that administration of a clinical adjuvant that signals TLR4 might treat patients with advanced disease receiving T cell–based immunotherapy. It is important to find an alternate agonist to LPS, however, because LPS cannot be realistically used in clinical trials of adoptive immunotherapy because of its inherent toxicity (110).

It is important to keep in mind that the expression and function of TLRs on immune cells in mice are different than humans (111). Thus, a variety of clinically available TLR agonists, such as virally derived imiquimod, that were ineffective in our hands (data not shown) might be effective in treating human patients, especially considering that imiquimod has been reported to treat some patients with superficial early-stage disease (112,113).

Future Directions: Alternate Reagents to Chemotherapy or TBI

Activation of the innate immune system via TLR4 signaling, removal of cytokine sinks, and depletion of MDSC and T_{reg} cells are key mechanisms underlying the effectiveness of TBI (Fig. 3). Based on these mechanisms, alternate reagents could be used in combination to enhance tumor destruction by adoptively transferred cells in humans. Alternate reagents to mimic these mechanisms are outlined in Table 1.

The patient's innate immune system might be activated with clinically relevant TLR agonists, including monophosphoryl lipid A, imiquimod, or CpG ODN 7909 (34,35,105–108,114–117). The CD40 costimulatory molecule, which is up-regulated on innate immune cells, such as dendritic cells, upon their activation might also enhance adoptive immunotherapy through engaging the CD40L molecule on adoptively transferred tumor-specific T cells. A recombinant human CD40 ligand could be used to mimic the activated innate immune cell, thereby enhancing the tumor-specific transferred T cells (118–123). Because combining TLR agonists have been reported to further activate the innate immune system, coadministration of these adjuvants might greatly enhance adoptive immunotherapy (124,125).

A number of approaches to eliminate human T_{reg} cells and cytokines sinks have been done with varying degrees of success, including ONTAK, Hu Max-CD4 (Zanolimumab), and RFT5 (126–133). Furthermore, B cells might act as sinks and/ or suppressor lymphocytes and thus

their removal with rituximab, a chimeric anti-CD20 monoclonal antibody, might also improve adoptive immunotherapy (134,135). Alternatively, homeostatic cytokines IL-7, IL-15, or IL-21 could be administered systemically to support the adoptively transferred cells because they are of low basal level in patient (136–143). Importantly, combining these reagents with clinical TLR agonists might greatly improve adoptive immunotherapy.

TLR Ligation May Foster the Development of Th17-like Cells

TLR agonists have been reported to reverse T_{reg}-mediated suppression on effector CD8 T cells, thereby enhancing their effector function (105,106,144). This blockade was partly dependent on IL-6 (produced by TLR-ligated APCs; ref. 144). However, emerging data indicate that IL-6 (together with transforming growth factor-β produced by activated APCs) also supports the development of CD4 IL-17 producing cells known as Th17 cells (145,146). Given the finding that Th17-like cells exacerbate autoimmune responses (ranging from uveitis, scleritis, arthritis to myocarditis) and are abundant in intestinal disease models (i.e., inflammatory bowel diseases such as colitis and Crohn's disease; refs. 147,148), it is possible that TLR ligation of translocated microbes might also fuel surviving and reconstituting CD4 cells to develop into Th17-like cells (149,150). Although the net effect of lymphodepletion-induced TLR4 ligation on surviving and reconstituting CD4 cells is less clear than its effect on T_{reg} cells and APCs, ablation might ultimately increase the antitumor reactivity of transferred T cells by increasing the activation and availability of Th17-like cells.

Conclusion

Lymphodepletion with chemotherapy or TBI enhances adoptive immunotherapy via several mechanisms. Beyond the removal of cytokine sinks and T_{reg} cells, translocation of gut microflora and especially of microbial-derived LPS by TBI can clearly affect the outcome of adoptive immunotherapy, a finding reminiscent of Coley's findings published >100 years ago.

Importantly, beyond a greater innate activation by gut microbes and more complete removal of inhibitory host cells (i.e., Foxp3^{+}CD4^{+} cells, CD8^{+} cells, and NK1.1^{+} cells) with a myeloablative preparative regimen, the HSC transplant promoted superior destruction of tumor by adoptively transferred CD8^{+} T cells (58). The efficacy of the transplant was attributed to the ability of the HSC to support the enhanced expansion and function of tumor-specific CD8^{+} T cells. Interestingly, however, little is known about the specific factors produced by HSC that improved the efficacy of the adoptively transferred cells. It is likely that the factors that influence HSC will be important for current T cell–based immunotherapy.

Although the tolerated doses of TBI and chemotherapy are well known, these systemic approaches are not devoid of toxicities. More targeted approaches that recapitulate the benefits of lymphodepletion may become valuable in the development of adoptive immunotherapy. The improved understanding of the mechanisms of action of TBI generates potential alternatives in the nonirradiated host, such as antibody-mediated lymphodepletion, administration of homeostatic cytokines, exogenous delivery of vaccine and/or TLR agonists, as well as HSC transplant in combination with adoptive immunotherapy (Table 1). The findings described in this review may therefore initiate novel treatment modalities for adoptive immunotherapy.

It is important to recognize the potential limitations of T cell–based immunotherapies. The extent to which tumor histologies other than melanoma will be susceptible to adoptive immunotherapy–based approaches is not yet known (151) and tumor cells are capable of escaping from T cell – based immunotherapies through a variety of mechanisms (152,153), included those described elsewhere in this *Clinical Cancer Research* Focus issue (154–157).

The ability to genetically engineer T cells promises to expand the histologies that will be susceptible to tumor destruction (40). The engagement of the innate immune system as a trigger of the adaptive immune system represents a powerful new approach as the field of the adoptive immunotherapy of cancer moves forward (90).

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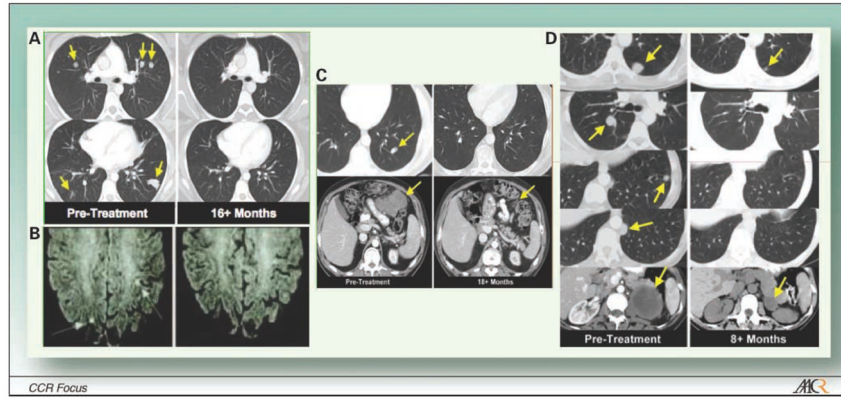
Fig. 1.
Tumor destruction of metastatic disease in various anatomic sites by adoptively transferred tumor-specific T cells in the setting of lymphodepletion with chemotherapeutic agents and/or TBI. Computed tomography scan of tumors in patients with stage IV melanoma. *A,* multiple large metastatic liver lesions existed before the treatment (pretreatment) and then regressed dramatically after adoptive T-cell transfer (16+ mo). *B,* patients with brain and recurrent axillary metastases experience tumor regression. *C,* multiple large metastatic liver lesions existed before the treatment (pretreatment) and then regressed dramatically after adoptive T-cell transfer (18+ mo) in patients receiving chemotherapeutic reagents in conjunction with 200 cGy TBI plus HSC transplant. *D,* multiple large metastatic liver lesions existed before the treatment (pretreatment) and then regressed dramatically after adoptive T-cell transfer (8+ mo) in patients receiving chemotherapeutic reagents in conjunction with six fractionated doses of 200 cGy TBI (twice a week for 3 d; 12 Gy TBI total) plus HSC transplant.
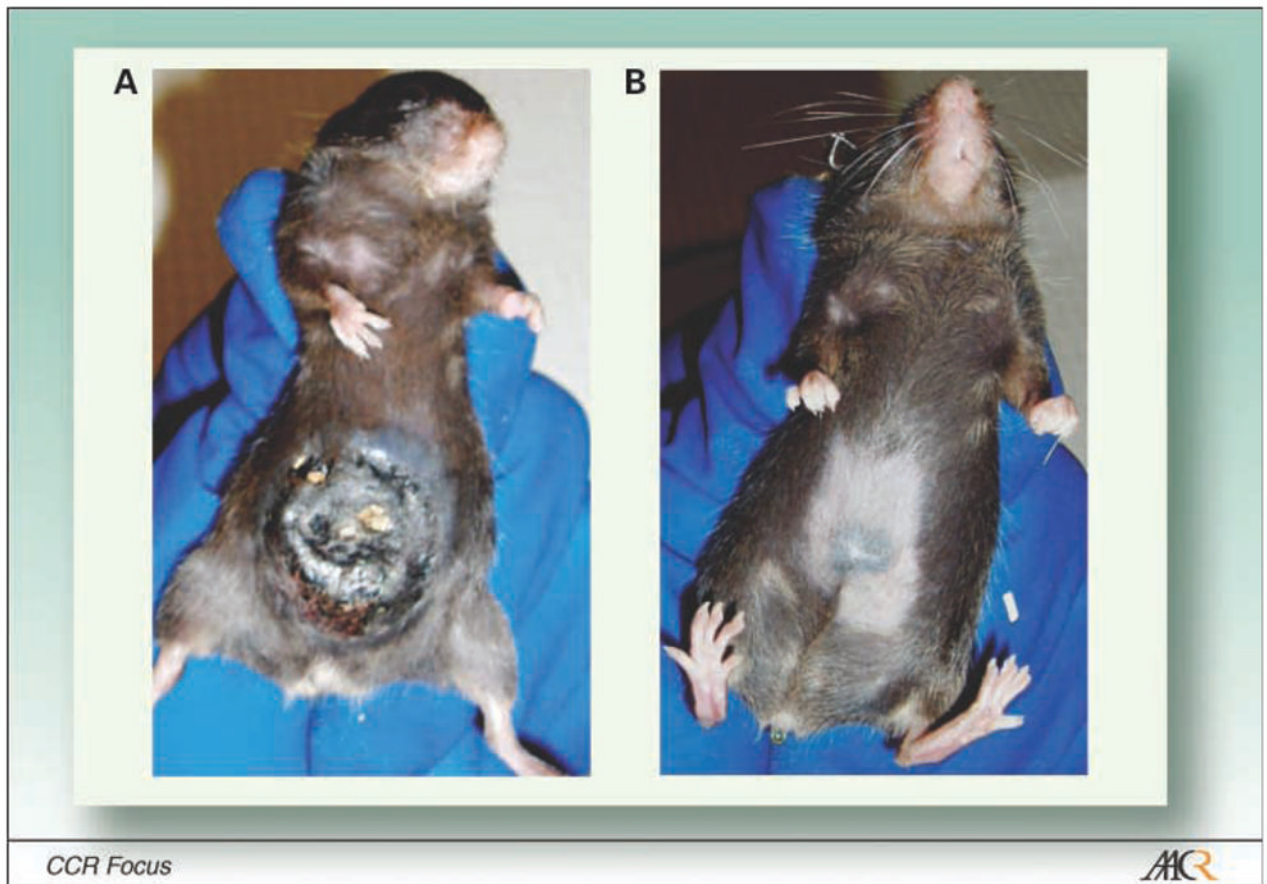
Fig. 2.
Tumor regression can be triggered by transgenic pmel-1Tcells. *A,* a mouse bearing a large, established, vascularized B16 mouse melanoma tumor shown after the start of treatment (19 d after tumor implantation). *B,* the same mouse (day 67).
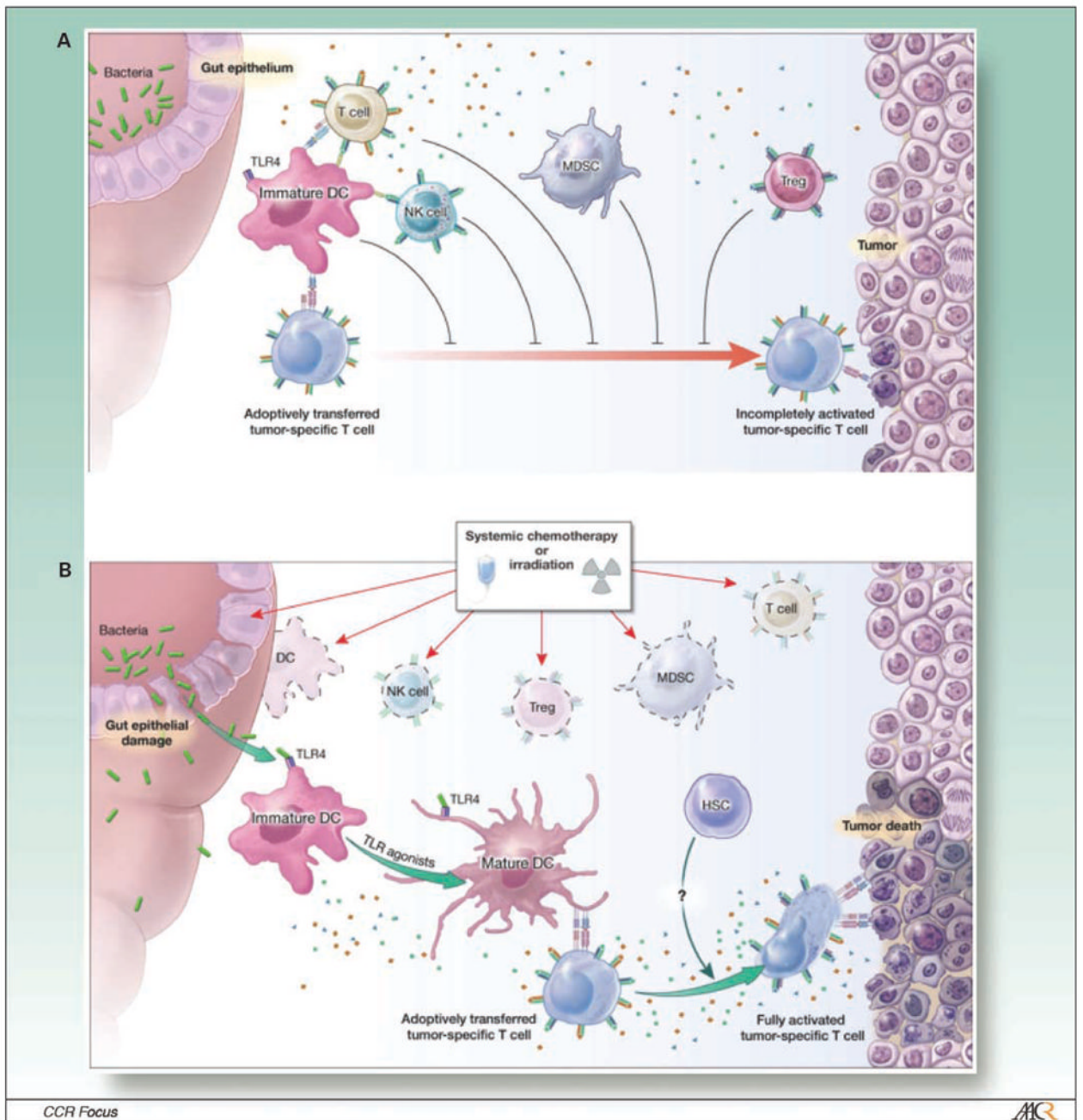
CCR Focus

Fig. 3.
Adoptive cell therapy in a lymphoreplete host. *A,* in a lymphoreplete environment, antitumor responses induced by adoptively transferred T cells are impaired because of their reduced availability of homeostatic cytokines (including IL-2, IL-7, and IL-15) by immune cells that consume them (cytokine sinks, which might include B cells,Tcells, and natural killer cells); and the suppressive activities of T$_{reg}$ cells, MDSCs, quiescent monocytes and dendritic cells (*DC*), and possibly natural killer cells. *B,* systemic chemotherapy or radiation before adoptive cell transfer alters the milieu of the tumor-bearing host. APCs are reduced in number by direct killing but there might be a net increase in lymphocyte activation because of reduced competition for antigen at the APC surfaces. At the same time, as a result of the translocation

of microbial LPS after chemotherapeutic or radiation-induced injury to the gut, dendritic cells mature and migrate to the lymph node via TLR4 signaling. This TBI action increases the functionality of the adoptively transferred lymphocyte. Activating γ-chain cytokines, such as IL-2, IL-7, and IL-15, as well as inflammatory cytokines, such as IL-6, IL-12, and tumor necrosis factor-α, are increased because of the removal of cellular sinks, T_{reg} cells, MDSCs, and natural killer cells, and concomitant innate immune activation. Collectively, these modifications promote the full activation of adoptively transferred T cells and tumor destruction.
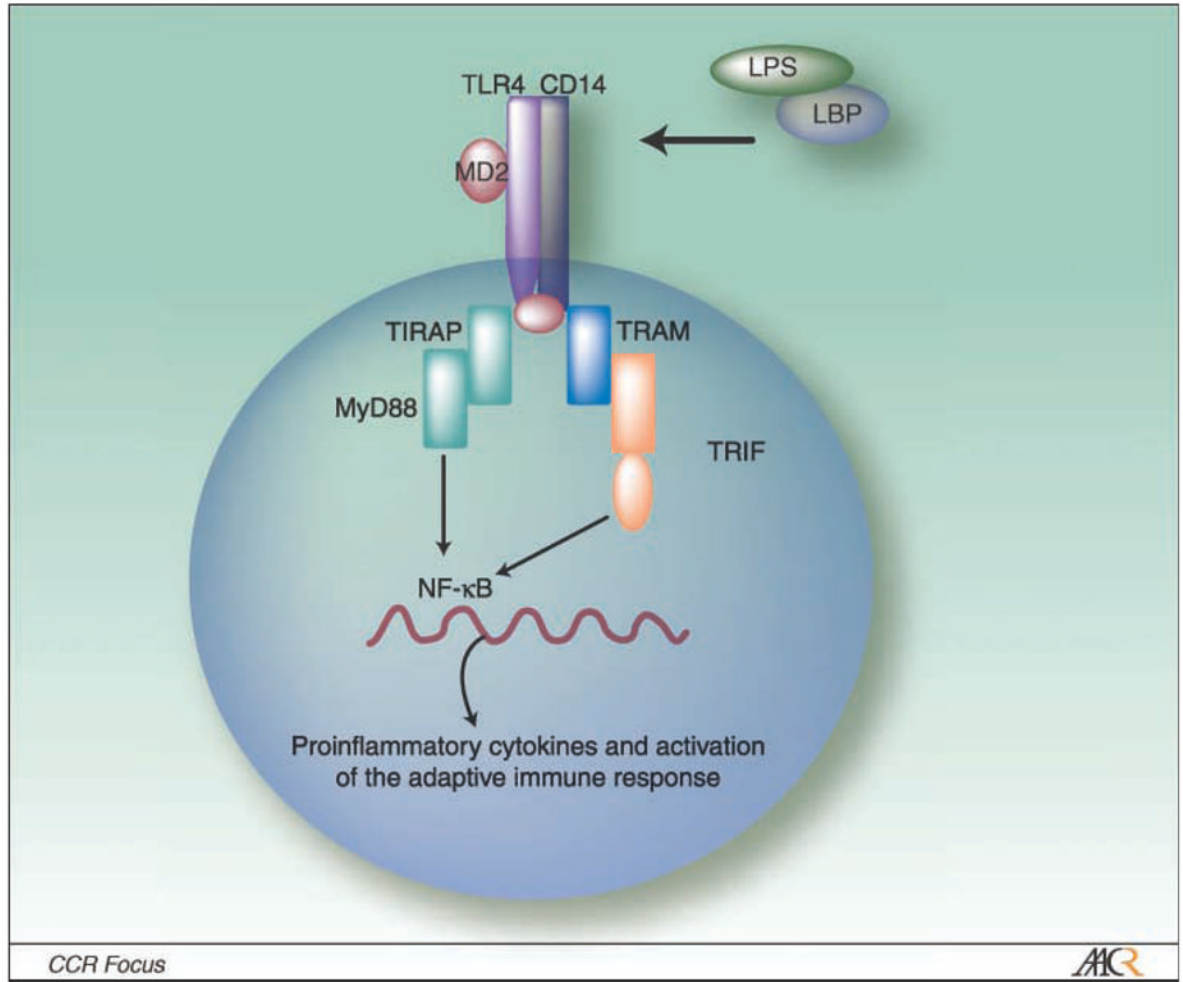
Fig. 4.
LPS signals the TLR4 pathway on an APC (i.e., monocyte or dendritic cell). TLR4 is a pathogen-associated receptor for Gram-negative bacterial product LPS.TLR4 requires MD-2, a secreted molecule, to functionally interact with the LPS:LPS binding protein complex (*LPB*). CD14 stabilizes and enhances LPS signaling. TLR4 engagement of LPS leads to nuclear factor-κB translocation via MyD88-dependent and MyD88-independent pathway that involve the TIR domain–ontaining adapter protein TIRAP. Adapted by permission from MacMillan Publisher Ltd: Nature Immunology 4; 1144–1150; Copyright 2003.

Table 1

Alternative clinical reagents that might mimic the effectiveness of lymphodepleting preparative regimens

Reagent	Mechanism of action
Depletion of regulatory elements and elimination of lymphocytes that consume homeostatic cytokines	
ONTAK	Denileukin diftitoxin
Humax-CD4	Human monoclonal antibody to CD4 molecule
RFT5-dgA	Recombinant immunotoxin to IL-2 receptor
LMB-2	Recombinant immunotoxin to Fv fragment of the anti-TAC anti-CD25 mAb
Rituximab	Depletes human B cells
Activation of the innate immune system	
MPL	TLR2/4
Imiquimod	TLR7 agonist
CpG ODN 7909	TLR9 agonist
Other reagents that might boost the expansion and/or function of adoptively transferred cells	
HSC	
Exogenous administration of IL-7, IL-12, IL-15, and/or IL-21	
CD40 ligation	Signal 2: T cells expressing CD40L engage CD40 molecule on APCs
CD28	Signal 2: T cells expressing CD28 engage B7 molecule on APCs