Arch. Immunol. Ther. Exp. (2010) 58:399–405
DOI 10.1007/s00005-010-0097-7

REVIEW

Tumor-Resident CD8$^+$ T-cell: The Critical Catalyst in IL-12-Mediated Reversal of Tumor Immune Suppression

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Received: 6 April 2010 / Accepted: 21 June 2010 / Published online: 26 September 2010

Abstract Tumor-resident T cells display a functionally impaired effector/memory (Tem) phenotype. Sustained intratumoral administration of IL-12, on the other hand, can restore cytolytic function to pre-existing CD8$^+$ Tem, resulting in effective tumor kill. Whereas cytotoxic T lymphocytes (CTL) are generally assumed to mediate tumor regression via direct tumor cytotoxicity, recent work revealed that activated CD8$^+$ Tem mobilize a systemic, multi-component effector cascade that includes both innate and adaptive immune mechanisms. Here we summarize these mechanisms, review how tumor-resident CD8$^+$ Tem orchestrate this cascade and discuss the potential clinical implications of these findings.

Keywords Tumor microenvironment · Immune therapy · CD8$^+$ T cells · Immune suppression · IL-12

Tumor Immune Suppression and Tumor-Infiltrating Leukocytes

The critical role of innate and adaptive immune mechanisms in controlling tumor progression is now well established (Dunn et al. 2004; Koebel et al. 2007). These findings have provided renewed impetus for the clinical development of immune-based therapies of cancer (Dougan and Dranoff 2009). At the same time both preclinical and clinical studies have demonstrated that tumors can continue to grow and metastasize in the presence of concomitant or induced immunity (Dunn et al. 2004; Koebel et al. 2007). Unchecked growth of tumors in the face of immune recognition has been attributed to multiple mechanisms including passive tumor immune evasion, active tumor immune suppression and/or immune exhaustion (Gajewski et al. 2006; Gajewski 2007; Whiteside 2008). In fact, tumors not only can neutralize antitumor immune effectors but also actively subvert inflammatory activity to their advantage (Johansson et al. 2008). The ability of tumors to grow despite florid leukocytic infiltration has prompted close scrutiny of the phenotype and functional properties of tumor-infiltrating leukocytes (TILs) in preclinical models and clinical samples (Lin and Pollard 2004; Mantovani et al. 2008; Oble et al. 2009; Talmadge et al. 2007). Such studies have demonstrated that these cells are either dysfunctional (Ahmadzadeh et al. 2009; Mantovani et al. 2008; Monsurró et al. 2004; Oble et al. 2009) or worse, display tumor-promoting inflammatory properties (Lin and Pollard 2004; Mantovani et al. 2008; Talmadge et al. 2007).

Tumor-Resident CD8$^+$ T Cells

The overwhelming majority of immune therapy studies focus on the induction of antitumor CD8$^+$ T cells due to their exquisite specificity and potent cytotoxicity (Mantovani et al. 2008). Yet, analysis of tumor-infiltrating T cells in murine tumor models and patient tumors has demonstrated that tumor-associated helper and cytotoxic T cells display a functionally impaired effector/memory phenotype (Ahmadzadeh et al. 2009; Mantovani et al. 2008; Monsurró et al. 2004). Monitoring of antitumor CD8$^+$ T-cell kinetics during tumor growth revealed that

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cytotoxic T lymphocytes (CTL) can effectively infiltrate and eliminate their targets during early tumor growth but lose cytotoxic function upon chronic exposure to the tumor microenvironment (Frey and Monu 2006; Huang et al. 2005; Janicki et al. 2008; Radoja et al. 2001). Specifically, tumor-associated $CD8^+$ T cells retained some ability to produce interleukin (IL)-2 and interferon (IFN)-γ but were completely defective in lytic function (Frey and Monu 2006; Huang et al. 2005; Janicki et al. 2008; Monsurró et al. 2004; Radoja et al. 2001). Loss of cytolytic ability was associated with a defective microtubule-organizing center, resulting in an inability to exocytose granules (Frey and Monu 2006; Radoja et al. 2001). These findings were consistent with the frequently observed lack of correlation between the post-therapy intensity of the antitumor $CD8^+$ T-cell response and efficacy of tumor regression in clinical trials (Rosenberg et al. 2005).

Molecular analysis of tumor-resident T cells traced defective cytolytic function to impaired T-cell receptor (TCR)-signaling in both murine (Frey and Monu 2008; Jarnicki et al. 1996; Koneru et al. 2005; Mizoguchi et al. 1992; Monu and Frey 2007) and human tumors (Agrawal et al. 1998, Broderick et al. 2006; Simpson-Abelson and Bankert 2008; Uzzo et al. 1999). Early studies revealed reduced levels of TCR CD3 ζ chain and $p56^{lck}$ kinase in tumor-resident and circulating T cells in tumor-bearing mice and patients, suggesting that downregulation or dissociation of these molecules was responsible for defective TCR-signaling (Jarnicki et al. 1996; Mizoguchi et al. 1992; Whiteside 2004). Other studies confirmed that T-cell dysfunction was associated with abortive proximal TCR-signaling but did not find significant deficits in the levels of the components of the signaling machinery (Frey and Monu 2008; Koneru et al. 2005). In these studies signaling deficiency was linked to the induction of Shp-1 phosphatase, activation of which resulted in dephosphorylation of $p56^{lck}$ and loss of lytic function (Frey and Monu 2008; Monu and Frey 2007). Further studies revealed that Shp-1 was activated by a tumor-dependent signal (Frey and Monu 2008). The exact nature of the tumor microenvironment-associated factor(s) that govern loss of proximal TCR-signaling and cytotoxic function in T cells is yet to be elucidated fully. Potential mechanisms include signaling via co-inhibitory molecules CTLA-4, PD-1 and BTLA (Peggs et al. 2008), which are upregulated on chronically stimulated T effectors (Mueller and Ahmed 2009; Peggs et al. 2008); the accumulation and persistence of suppressor cells (Treg and myeloid-derived suppressor cells, MDSC) and their byproducts such as immune suppressive cytokines transforming growth factor (TGF)-β and IL-10 plus the catabolic enzymes iNOS, arginase, and ectonucleotidase (Gajewski et al. 2006; Gajewski 2007; Whiteside 2008); and tumor-produced immune suppressive molecules including PD-L1, TGF-β, IL-10 and indoleamine 2,3 dioxygenase (IDO) among others (Gajewski et al. 2006; Gajewski 2007; Whiteside 2008).

TGF-β, which is produced by both tumors and tumor-infiltrating T-regulatory cells (Thomas and Massagué 2005; Shevach 2009), can have profound effects on TCR-signaling (Jarnicki et al. 1996; Li et al. 2006), including the induction of Shp-1 activity (Park et al. 2005). A critical role for TGF-β in defective T-cell signaling in human tumors was recently highlighted in *ex vivo* studies performed with T cells isolated from patient tumor samples (Broderick and Bankert 2006a; Simpson-Abelson and Bankert 2008). These studies demonstrated that acid elution of TGF-β from T cells resulted in rescue of TCR-signaling, whereas addition of TGF-β back to these cells restored signaling deficiency (Broderick and Bankert 2006a). Collectively, these findings point to an important role for the TGF-β-Shp-1-$p56^{lck}$ pathway in tumor-mediated T-cell dysfunction. It is however unlikely that TGF-β is the only factor that influences T-cell signaling in tumors. For example, intrinsic T-cell regulation via CTLA-4 (Peggs et al. 2008) or PD-1 (Blank and Mackensen 2007; Parry et al. 2005) as well as TCR nitrosylation by tumor-infiltrating MDSC (Bronte et al. 2003; Nagaraj et al. 2010) can result in downregulation of TCR-signaling. To this end, it should be noted that co-inhibitory molecule signaling via CTLA-4 and PD-1 involves Shp-1 activity (Peggs et al. 2008) and that TGF-β synergizes with these molecules in inhibiting TCR-signaling and promoting a regulatory phenotype in T cells (Francisco et al. 2009; Peggs et al. 2008). The specific contributions of these factors to T-cell dysfunction in the tumor microenvironment, and whether additional as yet unidentified factors are also involved, remains to be determined.

An important caveat to the above observations, particularly in the context of this article, is the nearly universal observation that tumor-associated T-cell dysfunction is reversible (Frey and Monu 2008; Mantovani et al. 2008; Monsurró et al. 2004). Indeed, when purified away from the tumor and incubated in culture both $CD4^+$ and $CD8^+$ T cells rapidly regain effector function (Frey and Monu 2008). To this end, tumor-infiltrating $CD8^+$ T cells are now routinely isolated and expanded *ex vivo* for adoptive cell transfer therapy (ACT) in melanoma patients (Rosenberg and Dudley 2009). While highly successful when combined with non-myelodepleting chemotherapy, the ACT approach has largely been limited to melanoma patients due to the relative ease of TIL isolation from these tumors but not others (Rosenberg and Dudley 2009). Moreover, the logistically challenging nature of this approach, including the need for the establishment of specialized facilities, has so far limited its clinical utility. In contrast,

a more clinically feasible approach may be offered by strategies that are designed to induce the activation and expansion of tumor-specific CTL in situ.

Restoration of Cytotoxic Function to $CD8^+$ Tem In Situ

Is it possible to overcome immune suppression in the tumor microenvironment and restore cytotoxic function to tumor-resident CTL in situ? At first look this is a daunting endeavor due to the multiplicity and potency of the suppressive mechanisms found in tumors. On the other hand, a number of studies have convincingly demonstrated that rescue of tumor-associated T-effector cytotoxicity is not only possible but in fact can lead to effective tumor eradication (Currie et al. 2008; Epardaud et al. 2008; Kilinc et al. 2006; Yu et al. 2005; Zang and Allison 2007). Two broad approaches have been evaluated. First, blocking or eliminating suppressor cells/co-inhibitory molecules has been used to rescue CTL activity (Dougan and Dranoff 2009; Peggs et al. 2008; Webster et al. 2007; Yu et al. 2005; Zang and Allison 2007). Second, direct activation of tumor-resident CTL with pro-inflammatory molecules such as TLR-ligands or cytokines has been employed to bypass immune suppression (Broderick et al. 2006; Currie et al. 2008; Dougan and Dranoff 2009; Epardaud et al. 2008; Kilinc et al. 2006). Whereas abrogation of suppressive mechanisms can result in CTL-mediated tumor regression, these strategies suffer from several drawbacks. For example, selective blocking of a single suppressor mechanism may be partially effective as suppressive pathways are numerous and redundant (Gajewski et al. 2006). Moreover, in many cases depletion of suppressors is non-specific and can also result in elimination of activated effectors (Dannull et al. 2005). Finally, complete and persistent blocking of soluble inhibitors may not be technically possible. In contrast, direct activation of effector T cells represents a more targeted and potentially feasible approach. Indeed, intratumoral delivery of pro-inflammatory molecules such as TLR ligands leading to pro-inflammatory cytokine release or direct delivery of such cytokines can rescue T-cell function independently of the existing suppressive mechanisms (Broderick et al. 2006; Currie et al. 2008; Epardaud et al. 2008; Kilinc et al. 2006).

Cytokine therapy of cancer has been evaluated extensively in the past three decades (Dranoff 2004). The early encouraging results obtained in preclinical models failed to translate to the clinical setting due primarily to the severe side effects associated with systemic delivery (Dranoff 2004). As a result, only two cytokines, IL-2 and IFN-α, have been approved for cancer therapy in metastatic melanoma and renal cancer (Dougan and Dranoff 2009). Intra- or peri-tumoral delivery of cytokines can circumvent the toxicity associated with systemic bolus delivery while maintaining efficacy (Brunda et al. 1993; Forni et al. 1985; Rook et al. 1999). However, this approach is not feasible for tumors that are difficult to reach as soluble cytokines require repeated injections due to rapid turnover (in the order of minutes). To this end, we have been testing the therapeutic potential of injectable sustained release cytokine formulations in the treatment of cancer (Egilmez et al. 2007). Our work has specifically focused on the antitumor potential of IL-12, a pro-inflammatory cytokine with potent NK and T-cell activating properties (Trinchieri 2003), which arguably represents the most effective cytokine for the treatment of advanced tumors (Cavallo et al. 1997). The therapeutic potential of IL-12 has been tested extensively in the clinic in the past 15 years (Del Vecchio et al. 2007). These studies have met with modest success due primarily to the severe side effects that are associated with bolus administration (Del Vecchio et al. 2007). In an attempt to circumvent systemic toxicity while maintaining efficacy, we have been evaluating the antitumor efficacy of intratumoral IL-12-encapsulated biodegradable micro/nanoparticles. These studies demonstrated that controlled-release IL-12 adjuvants were superior to soluble cytokine in eradicating established tumors in syngeneic murine and human/SCID mouse xenograft tumor models (Egilmez et al. 2000, 2002; Hill et al. 2002; Nair et al. 2006a; Sugiyama et al. 2001). Importantly, restoration of pre-existing tumor-resident Tem function was found to be central to the therapeutic efficacy of controlled-release intratumoral IL-12 adjuvants (Gu et al. 2008; Kilinc et al. 2006, 2009a, b).

Whereas the biology of IL-12, including the signaling pathways that mediate the T- and NK-cell proliferation and cytotoxic function, is well defined (Trinchieri 2003), how IL-12 overcomes impaired TCR-signaling in tumor-associated T cells has not been elucidated. The recent findings that IL-12 can override TGF-β signaling (Yu et al. 2006), particularly in the tumor microenvironment where defective TCR-signaling has been associated with TGF-β (Broderick et al. 2006; Broderick and Bankert 2006a; Park et al. 2005; Simpson-Abelson and Bankert 2008; Thomas and Massagué 2005), have shed light on this process. Moreover, IFN-γ, which is the primary downstream cytokine induced by IL-12, also inhibits TGF-β signaling, providing an additional mechanism for reversing TGF-β-mediated T-cell dysfunction (Higashi et al. 2003; Ulloa et al. 1999). Therefore, concurrent inhibition of TGF-β signaling and stimulation of cytotoxic mechanisms by the IL-12-IFN-γ axis likely underlies, at least in part, the IL-12-mediated rescue of tumor-resident T-cell function (Fig. 1). How IL-12 overcomes co-inhibitory molecule signaling in the tumor microenvironment, on the other hand, remains to be defined.

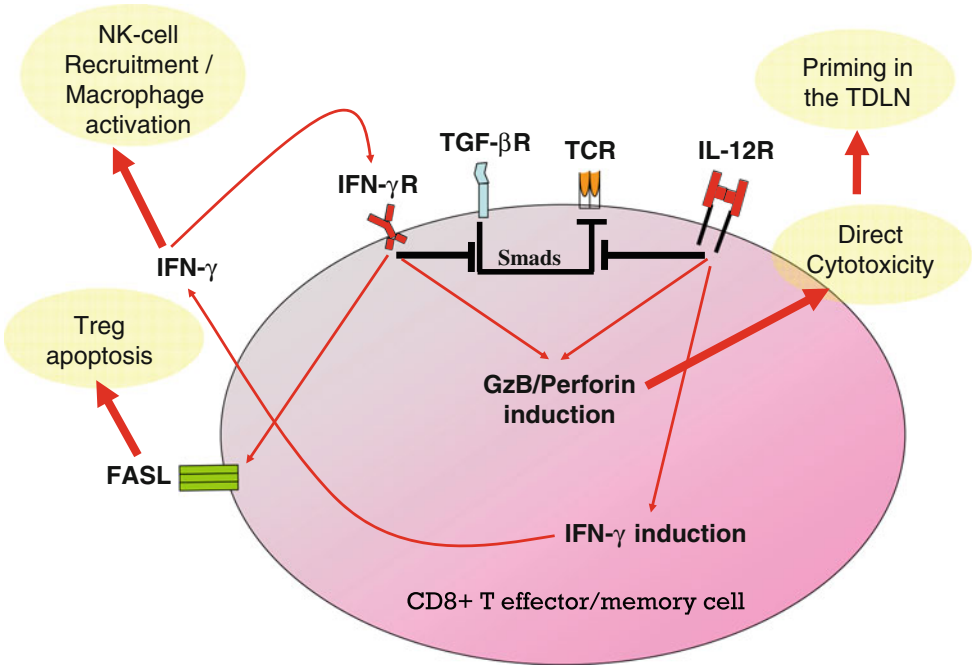

Fig. 1 IL-12-mediated rescue of tumor-resident $CD8^+$ Tem cytotoxicity results in the mobilization of multiple innate and adaptive antitumor effector mechanisms. Tumor-produced immune-inhibitory molecules, e.g. TGF-β, block TCR-signaling, leading to $CD8^+$ T-cell quiescence (*black lines*). Signaling through the IL-12 receptor inhibits Smads and restores TCR function. In addition, IL-12 induces IFN-γ and cytotoxic effector molecule production (*thin red arrows*). Autocrine IFN-γ further inhibits TGF-β signaling, promotes cytotoxic molecule production and upregulates FasL on $CD8^+$ Tem. Extrinsically, IFN-γ also promotes the activation and recruitment of innate effectors (NK cells and macrophages), while FasL mediates T-suppressor apoptosis (*thick red arrows*). Finally, local tumor kill and antigen release results in the priming of a secondary $CD8^+$ T-effector response in the tumor-draining lymph node (TDLN), which leads to systemic tumor cytotoxicity (*thick red arrow*)

The mechanisms outlined in Fig. 1 apply to both $CD4^+$ and $CD8^+$ T cells as IL-12 promotes similar rescue of both helper and cytotoxic T-cell function in the tumor microenvironment. The antitumor effector mechanisms mobilized by activated $CD4^+$ Tem have been described (Broderick and Bankert 2006b). The direct effects of IL-12 on intratumoral $CD8^+$ Tem function and the specific antitumor mechanisms that are marshaled by activated $CD8^+$ Tem are discussed below.

Effector Mechanisms Mobilized by Activated $CD8^+$ Tem

The most immediate effect of intratumoral IL-12 on tumor-resident T cells is the induction of IFN-γ by $CD4^+$ and $CD8^+$ Tem and restoration of cytolytic function to $CD8^+$ Tem (Kilinc et al. 2006). On the other hand, analysis of $CD8^+$ T-cell functional kinetics revealed that their activity was transient as the cells failed to expand and apoptosed rapidly within 3 days of activation (Kilinc et al. 2006). These findings, coupled with the fact that $CD8^+$ T cells comprise a very small proportion of TIL (Mantovani et al. 2008), suggest that IL-12-mediated restoration of lytic function to pre-existing $CD8^+$ Tem alone cannot explain the massive tumor destruction that occurs in post-therapy mice.

IL-12 also induces NK cell cytotoxicity and NK cells have been shown to play an important role in the eradication of both the primary and the metastatic tumors in IL-12 treated mice (Trinchieri 2003). Analysis of NK-cell infiltration and activation kinetics in tumors of post-IL-12 mice demonstrated that $CD8^+$ Tem activation was followed by a 3- to 6-fold increase in the number of intratumoral NK cells within 3 days of treatment (Gu et al. 2008). Importantly, NK-cell infiltration was strictly dependent on activation of pre-existing Tem and IFN-γ (Gu et al. 2008). Separately, others have demonstrated an important role for $CD8^+$ Tem in the activation of tumor-associated macrophages and macrophage-mediated killing of tumor in IL-12-treated mice (Tsung et al. 2002). These data establish that in addition to mediating direct cytotoxicity, tumor-resident T cells also promote the recruitment and activation of innate effectors in tumors.

The rescue of $CD8^+$ Tem function by IL-12 occurs despite the florid presence of fully functional $CD4^+$ $CD25^+$ $Foxp3^+$ T-suppressor cells in the tumor microenvironment (Kilinc et al. 2006). To determine whether IL-12

had an adverse effect on T-suppressor cell physiology, quantitative analysis of tumor-resident T-suppressors was performed (Kilinc et al. 2006). The results revealed a rapid sixfold drop in T-suppressor cell numbers concurrent with $CD8^+$ Tem activation (Kilinc et al. 2006). This unexpected finding led to further investigation of the mechanisms underlying the IL-12-mediated T-suppressor cell purge. These studies revealed that elimination of T-suppressors was associated with apoptotic death, which was directly mediated by activated $CD8^+$ Tem via the Fas/FasL extrinsic cell-death pathway (Kilinc et al. 2009b). Importantly, the upregulation of FasL on $CD8^+$ T cells was strictly dependent on autocrine production of IFN-γ by $CD8^+$ Tem, further clarifying the mechanism of IL-12-dependent elimination of T-suppressor cells from tumor (Kilinc et al. 2009b). These findings revealed a hitherto unknown role for $CD8^+$ T cells in controlling T-suppressor cell activity in tumors.

As mentioned above, the effector as well as the anti-suppressor activities of $CD8^+$ Tem were transient as these cells rapidly apoptosed (possibly via AICD) within 3 days of treatment (Kilinc et al. 2006). This represented a conundrum since tumor regression occurs primarily between days 7 and 14 post-treatment and thus one would expect antitumor cytotoxic activity to persist beyond the first 3–5 days. Long-term monitoring of intratumoral $CD8^+$ T-cell kinetics revealed that activation and apoptotic death of pre-existing $CD8^+$ Tem was followed by a second wave of $CD8^+$ T-effector cells, which infiltrated the tumors during days 4–10 post-treatment (Kilinc et al. 2006, 2009a). Further investigation demonstrated that the secondary $CD8^+$ T-effector response was primed *de novo* in the tumor-draining lymph node (TDLN) and, importantly, this priming was strictly dependent on the restoration of cytotoxic activity to pre-existing $CD8^+$ Tem (Kilinc et al. 2009a). Moreover, the primed cells not only homed to the primary tumor but also targeted distant metastatic nodules (Hill et al. 2002). These findings reveal the complex multi-component nature of the antitumor effector response that is orchestrated by IL-12 and identify the central role of pre-existing $CD8^+$ Tem in the initiation of this cascade.

Future Directions

The findings summarized above spotlight tumor-resident $CD8^+$ Tem as a potent in situ resource which, when activated fully, can rapidly restore local as well as systemic antitumor immunity. The advantages of targeting this population to jump-start antitumor immune activity are numerous. First, $CD8^+$ T-cell infiltrates are found in essentially all tumors. Second, this strategy involves minimal manipulation of patient tumors with no requirement for specialized facilities. Third, it does not require any knowledge or identification of tumor-associated antigens. Finally, re-activation of Tem not only restores direct tumor cytotoxicity but also mobilizes a cascade of secondary innate and adaptive effector mechanisms, reducing the probability of tumor escape.

Whereas the effector cascade that is mobilized by the IL-12/$CD8^+$ Tem axis promotes tumor regression, the overall window of antitumor cytotoxicity is still limited to 7–10 days. This interval appears to be sufficient to achieve complete eradication of small primary tumors (<4 mm) and micrometastatic disease in transplantable murine tumor models (Egilmez et al. 2000; Hill et al. 2002), but fails to achieve complete cure of advanced tumors in a spontaneous mammary tumor model (Nair et al. 2006a). Initial analysis of the mechanisms that impact the effector window revealed a dramatic rebound in intratumoral T-suppressor cell activity in post-therapy mice (Gu et al. 2010; Nair et al. 2006b). The counter-response was independent of tumor-immune suppression and intensified with repeated stimulation, ultimately resulting in a complete loss of therapeutic efficacy (Nair et al. 2006b). Further studies demonstrated that the regulatory rebound was associated with the IL-12-IFN-γ-indoleamine IDO axis (Gu et al. 2010). Specifically, IFN-γ-dependent upregulation of IDO in the tumors and the TDLN was found to be directly responsible for the post-therapy T-suppressor cell expansion (Gu et al. 2010).

The above studies identify treatment-induced feedback inhibition as a critical hurdle to tumor immune therapy. Therefore, T-cell based therapies, including those that aim to reactivate tumor-resident T cells, would be expected to benefit from concurrent administration of reagents that block counter-regulation. To this end, inhibition of IDO activity via administration of the IDO inhibitor 1-methyl tryptophan extended the $CD8^+$ T-effector cell activity window, resulting in superior tumor regression in post-IL-12 mice (Gu et al. 2010). Similarly, antibody-mediated blocking of T-cell intrinsic regulatory mechanisms, i.e. the co-inhibitory molecules CTLA-4 and PD-1, in mice vaccinated with cytokine-secreting tumor cells achieved durable tumor regression (Curran et al. 2010). Whether such strategies will be similarly effective in patients remains to be determined.

Acknowledgments This work was supported by the NIH grant 1-R01-CA100626 to N. K. Egilmez.

References

Agrawal S, Marquet J, Delfau-Larue M-H et al (1998) CD3 hyporesponsiveness and in vitro apoptosis are features of T

cells from both malignant and nonmalignant secondary lymphoid organs. J Clin Invest 102:1715–1723

Ahmadzadeh M, Johnson LA, Heemskerk B et al (2009) Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood 114:1537–1544

Blank C, Mackensen A (2007) Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother 56:739–745

Broderick L, Bankert RB (2006a) Membrane-associated TGF-beta1 inhibits human memory T cell signaling in malignant and nonmalignant inflammatory microenvironments. J Immunol 177:3082–3088

Broderick L, Bankert RB (2006b) Memory T cells in human tumor and chronic inflammatory microenvironments: sleeping beauties re-awakened by a cytokine kiss. Immunol Invest 35:419–436

Broderick L, Brooks SP, Takita H et al (2006) IL-12 reverses anergy to T cell receptor triggering in human lung tumor-associated memory T cells. Clin Immunol 118:159–169

Bronte V, Serafini P, Mazzoni A et al (2003) L-arginine metabolism in myeloid cells controls T-lymphocyte functions. Trends Immunol 24:302–306

Brunda MJ, Luistro L, Warrier RR et al (1993) Antitumor and antimetastatic activity of interleukin 12 against murine tumors. J Exp Med 178:1223–1230

Cavallo F, Signorelli P, Giovarelli M et al (1997) Antitumor efficacy of adenocarcinoma cells engineered to produce interleukin 12 (IL-12) or other cytokines compared with exogenous IL-12. J Natl Cancer Inst 89:1049–1058

Curran MA, Montalvo W, Yagita H et al (2010) PD-1 and CTLA-4 combination blockade expands infiltrating T cells and reduces regulatory T and myeloid cells within B16 melanoma tumors. Proc Natl Acad Sci USA 107:4275–4280

Currie AJ, van der Most RG, Broomfield SA et al (2008) Targeting the effector site with IFN-alphabeta-inducing TLR ligands reactivates tumor-resident CD8 T cell responses to eradicate established solid tumors. J Immunol 180:1535–1544

Dannull J, Su Z, Rizzieri D et al (2005) Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. J Clin Invest 115:3623–3633

Del Vecchio M, Bajetta E, Canova S et al (2007) Interleukin-12: biological properties and clinical application. Clin Cancer Res 13:4677–4685

Dougan M, Dranoff G (2009) Immune therapy for cancer. Annu Rev Immunol 27:83–117

Dranoff G (2004) Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 4:11–22

Dunn GP, Old LJ, Schreiber RD (2004) The immunobiology of cancer immunosurveillance and immunoediting. Immunity 21:137–148

Egilmez NK, Jong YS, Sabel MS et al (2000) In situ tumor vaccination with interleukin-12-encapsulated biodegradable microspheres: induction of tumor regression and potent antitumor immunity. Cancer Res 60:3832–3837

Egilmez NK, Hess SD, Chen F-A et al (2002) Human CD4+ effector T cells mediate indirect interleukin-12- and interferon-γ-dependent suppression of autologous HLA-negative lung tumor xenografts in severe combined immunodeficient mice. Cancer Res 62:2611–2617

Egilmez NK, Kilinc MO, Gu T et al (2007) Controlled-release particulate cytokine adjuvants for cancer therapy. Endocr Metab Immune Disord Drug Targets 7:266–270

Epardaud M, Elpek KG, Rubinstein MP et al (2008) Interleukin-15/interleukin-15Ralpha complexes promote destruction of established tumors by reviving tumor-resident CD8+ T cells. Cancer Res 68:2972–2983

Forni G, Giovarelli M, Santoni A (1985) Lymphokine-activated tumor inhibition in vivo. I. The local administration to interleukin 2 triggers nonreactive lymphocytes from tumor-bearing mice to inhibit tumor growth. J Immunol 134:1305–1311

Francisco LM, Salinas VH, Brown KE et al (2009) PD-L1 regulates the development, maintenance, and function of induced regulatory T cells. J Exp Med 206:3015–3029

Frey AB, Monu N (2006) Effector-phase tolerance: another mechanism of how cancer escapes antitumor immune response. J Leukoc Biol 79:652–662

Frey AB, Monu N (2008) Signaling defects in anti-tumor T cells. Immunol Rev 222:192–205

Gajewski TF (2007) Failure at the effector phase: immune barriers at the level of the melanoma tumor microenvironment. Clin Cancer Res 13:5256–5261

Gajewski TF, Meng Y, Harlin H (2006) Immune suppression in the tumor microenvironment. J Immunother 29:233–240

Gu T, Kilinc MO, Egilmez NK (2008) Transient activation of tumor-associated T-effector/memory cells promotes eradication via NK-cell recruitment: minimal role for long-term T-cell immunity in cure of metastatic disease. Cancer Immunol Immunother 57:997–1005

Gu T, Rowswell-Turner RB, Kilinc MO et al (2010) Central role of IFNgamma-indoleamine 2,3-dioxygenase axis in regulation of interleukin-12-mediated antitumor immunity. Cancer Res 70:129–138

Higashi K, Inagaki Y, Fujimori K et al (2003) Interferon-gamma interferes with transforming growth factor-β signaling through direct interaction of YB-1 with Smad3. J Biol Chem 278: 43470–43479

Hill HC, Conway TF Jr, Sabel MS et al (2002) Cancer immunotherapy with interleukin 12 and granulocyte-macrophage colony-stimulating factor-encapsulated microspheres: coinduction of innate and adaptive antitumor immunity and cure of disseminated disease. Cancer Res 62:7254–7263

Huang Y, Obholzer N, Fayad R et al (2005) Turning on/off tumor-specific CTL response during progressive tumor growth. J Immunol 175:3110–3116

Janicki CN, Jenkinson SR, Williams NA et al (2008) Loss of CTL function among high-avidity tumor-specific CD8+ T cells following tumor infiltration. Cancer Res 68:2993–3000

Jarnicki AG, Fitzpatrick DR, Robinson BW et al (1996) Altered CD3 chain and cytokine gene expression in tumor infiltrating T lymphocytes during the development of mesothelioma. Cancer Lett 103:1–9

Johansson M, Denardo DG, Coussens LM (2008) Polarized immune responses differentially regulate cancer development. Immunol Rev 222:145–154

Kilinc MO, Aulakh KS, Nair RE et al (2006) Reversing tumor immune suppression with intratumoral IL-12: Activation of tumor-associated T effector/memory cells, induction of T suppressor apoptosis, and infiltration of CD8+ T effectors. J Immunol 177:6962–6973

Kilinc MO, Gu T, Harden J et al (2009a) Central role of tumor-associated CD8+ T effector/memory cells in restoring systemic antitumor immunity. J Immunol 182:4217–4225

Kilinc MO, Rowswell-Turner RB, Gu T et al (2009b) Activated CD8+ T-effector/memory cells eliminate CD4+ CD25+ Foxp3+ T-suppressor cells from tumors via FasL mediated apoptosis. J Immunol 183:7656–7660

Koebel CM, Vermi W, Swann JB et al (2007) Adaptive immunity maintains occult cancer in an equilibrium state. Nature 450:903–907

Koneru M, Schaer D, Monu N et al (2005) Defective proximal TCR signaling inhibits CD8+ tumor-infiltrating lymphocyte lytic function. J Immunol 174:1830–1840

Li MO, Wan YY, Sanjabi S et al (2006) Transforming growth factor-beta regulation of immune responses. Annu Rev Immunol 24:99–146

Lin EY, Pollard JW (2004) Role of infiltrated leucocytes in tumour growth and spread. Br J Cancer 90:2053–2058

Mantovani A, Romero P, Palucka AK et al (2008) Tumour immunity: effector response to tumour and role of the microenvironment. Lancet 371:771–783

Mizoguchi H, O'Shea JJ, Longo DL (1992) Alterations in signal transduction molecules in T lymphocytes from tumor-bearing mice. Science 258:1795–1798

Monsurró V, Wang E, Yamano Y et al (2004) Quiescent phenotype of tumor-specific CD8 + T cells following immunization. Blood 104:1970–1978

Monu N, Frey AB (2007) Suppression of proximal T cell receptor signaling and lytic function in CD8 + tumor-infiltrating T cells. Cancer Res 67:11447–11454

Mueller SN, Ahmed R (2009) High antigen levels are the cause of T cell exhaustion during chronic viral infection. Proc Natl Acad Sci USA 106:8623–8628

Nagaraj S, Schrum AG, Cho H-I et al (2010) Mechanism of T cell tolerance induced by myeloid-derived suppressor cells. J Immunol 184:3106–3116

Nair RE, Jong YS, Jones SA et al (2006a) IL-12+ GM-CSF microsphere therapy induces eradication of advanced spontaneous tumors in her-2/neu transgenic mice but fails to achieve long-term cure due to the inability to maintain effector T-cell activity. J Immunother 29:10–20

Nair RE, Kilinc MO, Jones SA et al (2006b) Chronic immune therapy induces a progressive increase in intratumoral T suppressor activity and a concurrent loss of tumor-specific CD8+ T effectors in her-2/neu transgenic mice bearing advanced spontaneous tumors. J Immunol 176:7325–7334

Oble DA, Loewe R, Yu P et al (2009) Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human melanoma. Cancer Immun 9:3–22

Park I-K, Shultz LD, Letterio JJ et al (2005) TGF-β1 inhibits T-bet induction by IFN-gamma in murine CD4+ T cells through the protein tyrosine phosphatase Src homology region 2 domain-containing phosphatase-1. J Immunol 175:5666–5674

Parry RV, Chemnitz JM, Frauwirth KA et al (2005) CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms. Mol Cell Biol 25:9543–9553

Peggs KS, Quezada SA, Allison JP (2008) Cell intrinsic mechanisms of T-cell inhibition and application to cancer therapy. Immunol Rev 224:141–165

Radoja S, Saio M, Schaer D et al (2001) CD8+ tumor-infiltrating T cells are deficient in perforin-mediated cytolytic activity due to defective microtubule-organizing center mobilization and lytic granule exocytosis. J Immunol 167:5042–5051

Rook AH, Wood GS, Yoo EK et al (1999) Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. Blood 94:902–908

Rosenberg SA, Dudley ME (2009) Adoptive cell therapy for the treatment of patients with metastatic melanoma. Curr Opin Immunol 21:233–240

Rosenberg SA, Sherry RM, Morton KE et al (2005) Tumor progression can occur despite the induction of very high levels of self/tumor antigen-specific CD8+ T cells in patients with melanoma. J Immunol 175:6169–6176

Shevach EM (2009) Mechanisms of Foxp3+ T regulatory cell-mediated suppression. Immunity 30:636–645

Simpson-Abelson M, Bankert RB (2008) Targeting the TCR signaling checkpoint: a therapeutic strategy to reactivate memory T cells in the tumor microenvironment. Expert Opin Ther Targets 12:477–490

Sugiyama Y, Kato M, Chen F-A et al (2001) Human inflammatory cells within the tumor microenvironment of lung tumor xenografts mediate tumor growth suppression in situ that depends on and is augmented by interleukin-12. J Immunother 24:37–45

Talmadge JE, Donkor M, Scholar E (2007) Inflammatory cell infiltration of tumors: Jekyll or Hyde. Cancer Metastasis Rev 26:373–400

Thomas DA, Massagué J (2005) TGF-beta directly targets cytotoxic T cell functions during tumor evasion of immune surveillance. Cancer Cell 8:369–380

Trinchieri G (2003) Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat Rev Immunol 3:133–146

Tsung K, Dolan JP, Tsung YL et al (2002) Macrophages as effector cells in interleukin 12-induced T cell-dependent tumor rejection. Cancer Res 62:5069–5075

Ulloa L, Doody J, Massagué J (1999) Inhibition of transforming growth factor-beta/SMAD signaling by the interferon-gamma/STAT pathway. Nature 397:710–713

Uzzo RG, Rayman P, Kolenko V et al (1999) Renal cell carcinoma-derived gangliosides suppress nuclear factor-kappaB activation in T cells. J Clin Invest 104:769–776

Webster WS, Thompson RH, Harris KJ et al (2007) Targeting molecular and cellular inhibitory mechanisms for improvement of antitumor memory responses reactivated by tumor cell vaccine. J Immunol 179:2860–2869

Whiteside TL (2004) Down-regulation of zeta-chain expression in T cells: a biomarker of prognosis in cancer? Cancer Immunol Immunother 53:865–878

Whiteside TL (2008) The tumor microenvironment and its role in promoting tumor growth. Oncogene 27:5904–5912

Yu P, Lee Y, Liu W et al (2005) Intratumor depletion of CD4+ cells unmasks tumor immunogenicity leading to the rejection of late-stage tumors. J Exp Med 201:779–791

Yu J, Wei M, Becknell B et al (2006) Pro- and antiinflammatory cytokine signaling: reciprocal antagonism regulates interferon-gamma production by human natural killer cells. Immunity 24:575–590

Zang X, Allison JP (2007) The B7 family and cancer therapy: costimulation and coinhibition. Clin Cancer Res 13:5271–5279