Stable IL-10: A New Therapeutic that Promotes Tumor Immunity

Michele W.L. Teng,[1,2] Phillip K. Darcy,[1,2,*] and Mark J. Smyth[1,2,*]
[1]Cancer Immunology Program, Peter MacCallum Cancer Centre, St Andrews Place, East Melbourne, 3002 Victoria, Australia
[2]Department of Pathology, University of Melbourne, 3010 Victoria, Australia
*Correspondence: phil.darcy@petermac.org (P.K.D.), mark.smyth@petermac.org (M.J.S.)
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In this issue of *Cancer Cell*, Mumm et al. demonstrate that pegylated IL-10 increases CD8$^+$ T cell numbers, IFN-γ secretion, and cytotoxicity in established tumors, enhancing antigen presentation machinery and suppressing tumor growth. This approach may enhance T cell immune responses in cancers with reduced T cell infiltration.

IL-10 is an anti-inflammatory cytokine produced primarily by macrophages, regulatory T cells, and epithelial cells (Ouyang et al., 2011). IL-10 has pleiotropic effects in immunoregulation and inflammation, inhibiting the production of multiple, diverse inflammatory mediators (e.g., IL-12, MHC class II antigens and costimulatory molecules) from activated macrophages and dendritic cells—a process requiring STAT3 activation. It also downregulates the expression of Th1 cytokines (such as IFN-γ, TNF, IL-1β, and IL-6) and impairs secondary CD8$^+$ T cell responses. More generally, IL-10 enhances B cell survival, proliferation, and antibody production. IL-10 deficiency in mice suggests the function of this cytokine as an essential immunoregulator in the intestinal tract, and mutations in IL-10 are also associated with an increased susceptibility to HIV-1 infection and rheumatoid arthritis.

The dual functions of IL-10 and IFN-γ in antitumor immunity and immunoregulation have been recognized for some time (Wilke et al., 2011). It must be said that much confusion has surrounded the biological function of cytokines in tumor immunity because the effects of neutralizing or deleting an endogenous cytokine are often quite distinct from those when the same cytokine is exogenously administered or ectopically-expressed from the tumor. IL-23 is a case in point where blockade or deletion of the host cytokine leads to reduced tumor incidence (Teng et al., 2010), yet paradoxically, exogenous or ectopic cytokines also cause tumor regression (Lo et al., 2003). Here, however, Mumm et al. (2011; this issue of *Cancer Cell*) present a series of quite concordant results indicating that host IL-10 and exogenous pegylated IL-10 (PEG-IL-10) promote CD8$^+$ T cell control of developing or established tumors, respectively. The data regarding therapeutic PEG-IL-10 are novel and convincing. Mumm et al. (2011) clearly show that PEG-IL-10 increases CD8$^+$ T cell numbers in large transplanted and spontaneous tumors and reduces tumor size (Figure 1). It is unclear from the results presented whether PEG-IL-10 increases T cell infiltration or increases survival or proliferation of T cells in the tumor—IL-10 has been shown to do the latter. The results showing suppression of large HER2-driven mammary cancers by PEG-IL-10 are quite remarkable for a single therapy. In correlation, PEG-IL-10 directly induces granzymes and IFN-γ in CD8$^+$ T cells and antigen presentation indirectly via CD8$^+$ T cell-derived IFN-γ. Mumm et al. (2011) also show that IL-10 expression correlates with the expression of granzymes, IFN-γ, and MHC molecules in human tumors. The data suggest that PEG-IL-10 might not be very effective for tumors that lack responsiveness to IFN-γ, a well-characterized immune escape mechanism.

IL-10-deficient mice are known to develop colitis and colorectal cancer in a strain dependent manner. In concert, in the colitis and skin papilloma resistant C57BL/6 strain, Mumm et al. (2011) clearly observed that IL-10-deficient mice were more sensitive to DMA/TPA-induced papilloma, whereas human IL-10 transgenic mice were more resistant. These data are consistent with an important role for host IL-10 in controlling tumor initiation but are difficult to reconcile with some other studies using this carcinogen-induced skin tumor model. Notably, innate $\gamma\delta$ T cells have been shown to be critical host protective effector cells in the DMBA/TPA-induced skin papilloma model, rather than CD8$^+$ $\alpha\beta$ T cells (Girardi et al., 2003). Furthermore, Xiao et al. (2009) have shown in the 129 strain that host IFN-γ promotes papilloma development. Both of these previous findings are inconsistent with Mumm et al's major hypothesis that IL-10 reduces skin and other tumor development via CD8$^+$ T cell and IFN-γ-dependent mechanisms. One has to be cautious in making general conclusions from mouse models that are strain-dependent. In a larger series of studies performed using methylcholanthrene, a carcinogen that induces fibrosarcomas, host IL-10 was shown to promote tumor formation (Swann et al., 2008). To date, there has been no report linking IL-10 and IL-10RA expression as a positive correlate for survival of cancer patients. In a large cohort of colorectal cancer patients where effector memory T cells correlate tightly with disease stage and improved prognosis, there was no correlation between expression of IL-10 and IL-10 receptor and a positive prognosis (Jerome Galon, personal communication). However, it will be important in future studies to clarify whether a positive correlation exists in other patient cohorts, which would strengthen the clinical relevance of IL-10 and its role in cancer immunity. Although it is not trivial, assessing conditional deletion of IL-10 in many other mouse models of cancer might yield a clearer picture of the role

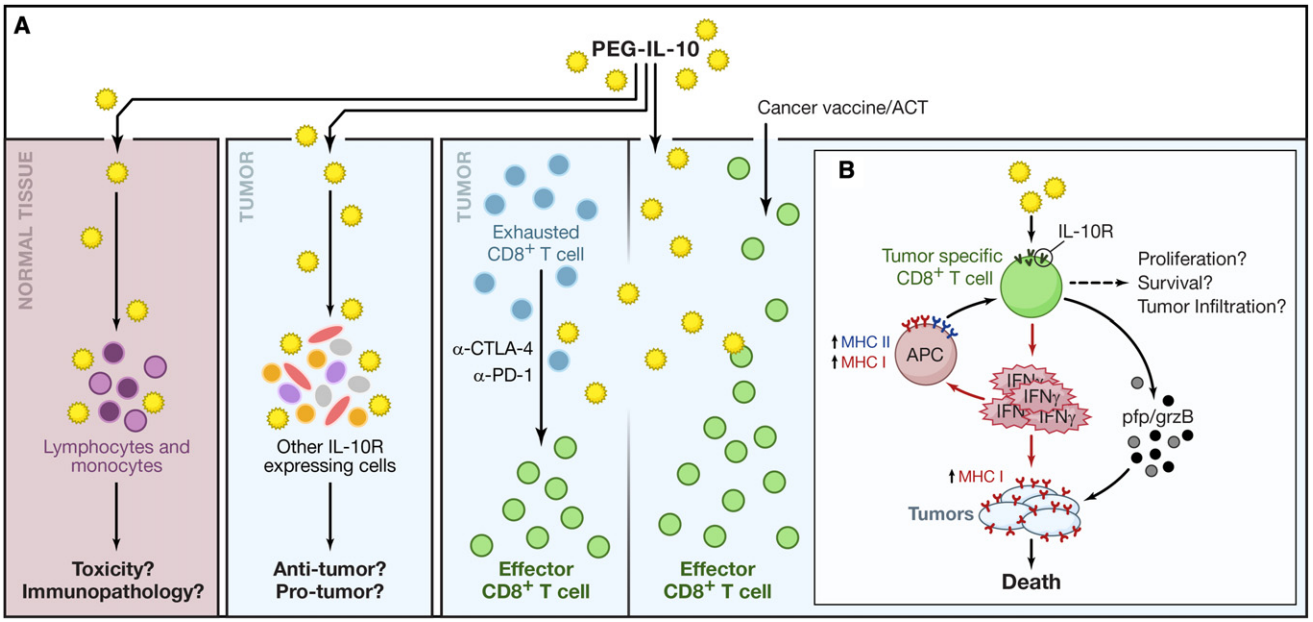

Figure 1. A New IL-10 Cancer Therapeutic

The study by Mumm et al. (2011) proposes that treatment with PEG-IL-10 may be effective in increasing $CD8^+$ T cell numbers in the tumor microenvironment. (A) Systemic PEG-IL-10 may bind either $CD8^+$ T cells (middle right) or other IL-10R leukocytes (middle left) in the tumor microenvironment. Following an increase in intratumor $CD8^+$ T cell numbers by a number of possible mechanisms detailed in inset (B), the boosted adaptive immune system may combine well with other immunotherapeutic strategies such as cancer vaccination/adoptive cellular transfer (ACT) to enhance effector $CD8^+$ T cell numbers or anti-CTLA-4 or anti-PD-1 blockade of T cell checkpoint molecules to convert exhausted $CD8^+$ T cells into effector $CD8^+$ T cells in the tumor microenvironment (middle right). The antitumor or protumor effects of PEG-IL-10 via other IL-10R-expressing cells remain unclear (middle left). PEG-IL-10 therapy may also lead to lymphocyte and monocyte infiltration and varying degrees of toxicity/immunopathology in different normal tissues (left panel). Inset (B) in the tumor, PEG-IL-10 binds IL-10R more highly expressed on $CD8^+$ T cells triggering perforin (pfp)/granzyme B (grzB) production and IFN-γ release. These may act directly on tumor cells or IFN-γ indirectly by enhancing tumor MHC class I or antigen presentation (via MHC class I and II) on antigen presenting cells (APC).

of host IL-10 in cancer initiation and development.

Mumm et al. (2011) showed that IL-10 directly induced cytolytic molecules in $CD8^+$ T cells and antigen presentation indirectly through $CD8^+$ T cell-derived IFN-γ. However, other studies have reported that administration of IL-10 can result in increased NK cell-mediated rejection of metastases independently of T cells (Zheng et al., 1996). Given that IL-10RA is expressed on a variety of different immune cells including NK cells, CD4 T cells, neutrophils, monocytes, and macrophages, IL-10 could potentially induce antitumor responses through many different cellular immune subsets. This further highlights the need to determine whether other immune subsets can contribute to IL-10-mediated rejection in a number of clinically relevant tumor models.

The central tenet by Mumm et al. (2011) is that T cell polarization and the effector response, rather than immune recognition per se, are deregulated by tumors. They postulate that redirecting T cells in tumor pathology into cytotoxic effectors may represent a powerful new immunotherapeutic approach against late stage cancer. Their current work raises the interesting possibility that new combination strategies may be designed to capitalize on the adaptive tumor-specific immunity generated by PEG-IL-10. In particular, the ability of PEG-IL-10 to increase tumor infiltration of $CD8^+$ T cells should effectively combine with approaches, approved or in late phase clinical trials, that enhance the function of tumor-specific $CD8^+$ T cells that have reached the tumor but fail to act (Figure 1). Such therapeutics might include antibodies targeting CTLA4, PD-1/PD-L1, TIM3, or CD137. Indeed, it will be interesting to determine the level of expression of these proteins on tumor-specific and infiltrating T cells following PEG-IL-10 therapy. In addition, some cancer vaccines that stimulate decent systemic numbers of tumor-specific T cells might benefit when combined with PEG-IL-10. One decade ago, a report elegantly showed that injection of IL-10 just after a booster vaccine significantly enhanced antitumor immunity (Fujii et al., 2001). The combination of each of these approaches with PEG-IL-10 must now be tested in preclinical mouse models of cancer.

A very important consideration for the translation of PEG-IL-10 into the clinic is potential toxicities and immunopathologies that might be caused by this formulation. The authors reported that PEG-IL-10 mediated some lymphocyte and monocyte infiltrations and apoptosis of epithelial cells in organs such as the liver and pancreas. If more generalized, these pathologies may be quite dose-limiting and concerning. The authors do show an increased IL-10RA expression on tumor-infiltrating $CD8^+$ T cells compared with other immune cells, but this difference was quite modest. Given concerns over the safety profile of some other immunotherapeutics, mechanisms underlying the toxicities of PEG-IL-10 require closer scrutiny.

In summary, PEG-IL-10 may represent a new avenue to improve oncology outcomes, and the debate concerning

the immunostimulatory versus immunosuppressive effects of IL-10 remains alive and well.

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"Ring-Fencing" BRCA1 Tumor Suppressor Activity

Ketan J. Patel,[1,*] Gerry P. Crossan,[1] and Michael R.G. Hodskinson[1]
[1]MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 0QH, UK
*Correspondence: kjp@mrc-lmb.cam.ac.uk
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BRCA1 is a crucial human breast and ovarian cancer tumor suppressor gene. The article by Drost et al. in this issue of *Cancer Cell* together with a recent paper in *Science* now provide a clearer picture of how this large and complex protein suppresses tumorigenesis.

Breast and ovarian cancer are major causes of mortality and morbidity in the developed world. Up to 10% of all breast cancers are due to the inheritance of germline mutations in two breast cancer susceptibility loci (*BRCA1* and *BRCA2*). In fact, mutations in *BRCA1* account for up to 80% of families with breast and ovarian cancer predisposition and therefore pose a significant burden to human health. The *BRCA1* gene encodes a large polypeptide that interacts with its constitutive binding partner BARD1. There is a body of evidence indicating that the BRCA1-BARD1 heterodimer is a crucial regulator of the cellular response to DNA damage. Loss of BRCA1 results in genomic instability, probably due to an impaired DNA damage response. It is therefore likely that BRCA1 suppresses tumorigenesis by preventing genetic instability.

Two regions of the BRCA1 protein are thought to be critical to this function: first, an N-terminal RING domain that has E3 ubiquitin ligase activity that is potentiated through its interaction with BARD1; and second, the C-terminal BRCT domain that mediates the specific interaction with the phosphorylated form of DNA repair factors (Figure 1) (Huen et al., 2010). However, the mechanism by which these two regions contribute to tumor suppression has not been clarified. Two recent papers provide this critical information (Shakya et al., 2011; Drost et al., 2011 [this issue of *Cancer Cell*]). In addition the conclusions drawn from these new studies have potential clinical implications for the treatment of patients with breast cancer in which *BRCA1* has been mutated.

Using mice, Shakya et al. (2011) dissected the function of the RING domain associated ubiquitin ligase activity and the BRCT domain. In particular they assessed how these two regions of BRCA1 contributed to embryonic development (the homozygous *Brca1* null mutation is embryonic lethal) and tumor suppression. The E3 ligase activity of the RING domain has previously been implicated in the DNA damage response (Ruffner et al., 2001) and more recently in the maintenance of heterochromatin (Zhu et al., 2011). Furthermore, human cancer predisposing mutations often clustered in this region with some, such as $BRCA1^{C61G}$, abrogating E3 ubiquitin ligase activity. Cellular studies have shown that BRCA1 localized to DNA damage induced foci which also contained polyubiquitinated substrates (Morris and Solomon, 2004). Though compelling, these lines of evidence are correlative and lack genetic evidence directly linking the E3 ubiquitin ligase activity to the role of BRCA1 in DNA repair and in tumor suppression. Shakya et al. (2011) therefore engineered a point mutation within the RING finger domain of BRCA1. This $Brca1^{I26A}$ mutation results in the loss of the E3 ligase activity of BRCA1 but does not compromise either the stability of the protein or its interaction with BARD1. Previous work from the