T-cell transmigration, and this effect was inhibited by galardin, suggesting MMP involvement.

The same authors recently published a study in which they demonstrated that IL-13, another mediator derived from mast cells and T helper 2 cells, can also induce MMP-9 in keratinocytes, promote collagen type IV degradation, and increase T-cell transmigration through Matrigel. However, neither additive nor synergistic MMP-9 expression could be detected when IL-13 acted together with TGF-β or TNF-α (Purwar et al., 2008).

Concluding remarks
The present study provides interesting insights into the role of histamine in skin inflammation and epidermal pathology. In fact, a variety of mast cell mediators, including histamine, IL-13, TNF-α, and TGF-β, can evidently stimulate keratinocytes for MMP-9 expression (Figure 1). Furthermore, different combinations of these mediators can even lead to enhanced proteinase expression. Nevertheless, it must be clarified whether this histamine-induced MMP-9 expression, and possibly also induction of other MMPs, together with subsequent basement membrane degradation and T-cell transmigration to the epidermis, actually take place in vivo in pathologic processes, such as psoriasis, eczema, or skin blistering, with resultant epidermal inflammation. It is noteworthy that histamine can also simultaneously induce numerous other molecules in keratinocytes and that these molecules can also impact immunopathogenesis. Because histamine can act together with TNF-α, IFN-γ, and TGF-β, leading to an enhanced biological effect, it is tempting to speculate that antihistamines might enhance the therapeutic effects of TNF-α-blocking drugs (Cordiali-Fei et al., 2006).

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Tumor Evasion May Occur via Expression of Regulatory Molecules: A Case for CTLA-4 in Melanoma
Jeffrey S. Weber

Emerging data that show immune-regulating molecules may be ectopically expressed on human tumors suggest that novel mechanisms may induce tumor-related immune suppression, and support the use of antibodies and small molecules to target those immune-suppressive moieties.

In the words of Oscar Wilde, “To expect the unexpected shows a thoroughly modern intellect.” In this issue of the Journal of Investigative Dermatology, Shah et al. (2008) describe the upregulation of a variety of genes induced in melanoma cell lines by Wnt-β-catenin signaling. Surprisingly and unexpectedly, cytotoxic T-lymphocyte antigen-4 (CTLA-4), an immune regulatory molecule, emerged as the gene product most highly increased in the authors’ transcripational profiling and expression array analysis of melanoma cell lines treated with recombinant Wnt 3a. CTLA-4 is a molecule expressed on T cells that delivers a signal decreasing T-cell activation by the recruitment of tyrosine and serine/threonine phosphatases (Walunas et al., 1994; Kimmel and Allison, 1996). CTLA-4 is clearly inhibitory for T-cell activation because lymphoproliferative/autoimmune events are observed in CTLA-4−/− mice.

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4H. Lee Moffitt Cancer Center & Research Institute, Tampa, Florida, USA

Correspondence: Jeffrey S. Weber, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, SRB-2, Tampa, Florida 33612, USA. E-mail: jeffrey.weber@moffitt.org

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In patients, the abrogation of CTLA-4 activity with human monoclonal antibodies leads not only to autoimmune side effects but also to sustained tumor regression or stable disease in selected patients with advanced melanoma. The unexpected presence of CTLA-4 on tumor cell lines and in the analysis by Shah et al. of early-stage and metastatic melanoma tumors is consistent with data from at least one prior publication on this topic, but it nicely expands the emerging story of how expression of “ectopic” immune regulatory molecules by tumor cells may alter the balance between immune stimulation and inhibition. CTLA-4 expression by tumors may represent yet another mechanism by which tumors actively suppress, evade, and avoid T-cell immunity and exhibit immune “escape.”

Immune regulatory molecules have previously been demonstrated to be expressed on a variety of human and murine tumors. B7-1, or CD80, is a molecule commonly found on activated antigen-presenting cells such as dendritic cells, B cells, and macrophages and has been found on human colon tumors at a low level, resulting in immune downmodulation (Tirapu et al., 2006). A dichotomous relationship of B7-1 likely exists, because high levels of CD80 or CD86 on tumor cells driven by gene engineering increase, rather than decrease, their immunogenicity. CD80 and the similar costimulatory molecule CD86 have also been reported at low levels on a broad variety of tumor cell lines by flow cytometry analysis and reverse transcriptase PCR (Contardi et al., 2005). Programmed death ligand-1 (PD-L1, or B7-H1) is another molecule expressed on activated human antigen-presenting cells and is commonly found on melanomas, as well as in colon, breast, and lung cancers. PD-L1 acts as a further “brake” on immune activation, similarly to CTLA-4. High levels of PD-L1 bind to the PD-1 receptor on T cells and limit T-cell proliferation, function, and activation via the T-cell receptor (Dong et al., 2002). Levels of PD-L1 on tumors correlate with a poor outcome in ovarian and other human cancers (Hamanishi et al., 2007). Finally, the co-stimulatory molecule 4-1BB, like other members of the tumor necrosis factor receptor superfamily, is also found at low levels on colon and other cancer cells and can exert a functional effect when cells expressing 4-1BB are treated with its soluble ligand (Salih et al., 2000). These data suggest a theme by which tumors can evolve to an immune evasion phenotype and inhibit adaptive and innate immunity by exploiting naturally expressed immune regulatory molecules, and they suggest strategies to overcome this phenomenon as well as possible new predictive markers for the success or failure of current immunoregulatory antibody treatments.

Prior studies have demonstrated that CTLA-4 is expressed not only on activated T cells but also on cells of the mononuclear lineage, cultured muscle cells, and placental fibroblasts. CTLA-4 has previously been demonstrated on melanoma cell lines; a variety of other human tumor cell lines such as osteosarcoma, neuroblastoma, and infiltrating breast cancer lines; and lymphoid and myeloid leukemia cells (Contardi et al., 2005). CTLA-4 was found on sarcoma and breast cancer tissue, as demonstrated by immunohistochemical staining. Interestingly, when soluble B7.1 or B7.2 was used to treat the tumor lines expressing CTLA-4 in the above work, apoptosis induced by increases in cleaved caspases 3 and 8 was observed, suggesting that the tumor cell–derived CTLA-4 was functional.

The targets of Wnt signaling include genes of the melanocytic pathway such as MITF, as well as lymphoid-related genes. Signaling through Wnt clearly plays a role in melanocytic development and may be a marker of aggressive behavior in melanoma via an interaction with the protein kinase C pathway (Weeraratna et al., 2002). In their analysis in the current work, Shah et al. (2008) demonstrate that CTLA-4 was present not only on melanoma cell lines but also on human epidermal melanocytes and melanoma tumors using flow cytometry staining. The expression on the melanoma cell lines and the melanocytes was clearly increased by Wnt-3a and represented full-length (not truncated) RNA transcripts. However, the actual surface expression of CTLA-4 seemed unchanged, suggesting that intracellular, nonfunctional CTLA-4 was actually modulated by Wnt-a signaling. The data are admittedly derived from only two cell lines and may not be representative of all melanomas; in addition, as opposed to work performed previously, no functional studies were undertaken to demonstrate that physiologic consequences might arise from Wnt-induced CTLA-4 expression by melanoma cell lines, so some caution in interpreting the results of this work is indicated. Nonetheless, the experiments described in this work are of importance and require follow-up, because even low levels of extracellular CTLA-4 in the presence of high levels of intracellular material can be functional (Contardi et al., 2005).

What is the implication of this work and the work of others cited above? First, the results indicate that tumor cell expression of immune regulatory molecules might be more widespread than previously thought and may be yet another important immune-suppressive mechanism in humans. Alternatively, we may need to expand our concept of CTLA-4 as an immune-modulating molecule and ask whether it might have other functions in the regulation of nonlymphoid cells under the control of the Wnt family. Recent clinical trials with two different CTLA-4 abrogating antibodies have indicated that they have clinical activity against melanoma and other malignancies. If the results of ongoing large phase III trials of ipilimumab, a human IgG1 anti-CTLA-4 antibody from Bristol-Myers Squibb, are positive, then a more extensive analysis of tumors from patients who received the antibody is in order to assess whether clinical benefit might correlate with tumor-related CTLA-4 expression.

Could other molecules that are traditionally thought of as having immunity-regulating activity also
function in a nonimmune milieu, like PD-L1, a suppressive influence, and 4-1BB, an immunity-stimulating receptor? The ability to quantitate molecules such as CTLA-4 on and within tumors by immunohistochemical staining or their detection as soluble molecules in the serum of cancer patients merits their exploration as prognostic markers, and even predictive markers, for therapy using antibodies to CTLA-4 and other immunity regulatory molecules in development. Finally, the clearest therapeutic implications of the current work are the indication of a new mechanism for the utility of CTLA-4-abrogating antibodies and the suggestion that using a non-toxic pretreatment to upregulate those molecules on melanomas might provide a synergistic mechanism by which the utility of the antibodies may be augmented. New small-molecule synergists of Wnt signaling are becoming available, and they warrant testing in a strategy to add to the antitumor activity of CTLA-4 abrogation (Zhang et al., 2007). Shah et al. (2008) have demonstrated a thoroughly modern intellect by documenting the unexpected expression of CTLA-4 on melanoma and its upregulation by Wnt signaling.

CONFLICT OF INTEREST
The author states no conflict of interest.

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