

◀ **Close contact.** MDSCs (red) are shown here interacting with CD8<sup>+</sup> T cells (magenta).

tion's carcinogenic effects.

Some clinical implications of these findings are already beginning to emerge. As cancer immunologists learn what makes MDSCs tick, they are using that information to design strategies to counteract them in the hope that this will make anticancer vaccines and other immunotherapies more effective. Indeed, researchers have already identified drugs that inhibit MDSCs and have begun preliminary clinical trials. **If what the field has learned so far is correct, "using different drugs [to block MDSC action]**

**could drastically improve responses to cancer vaccines,"** predicts Dmitry Gabrilovich of the H. Lee Moffitt Cancer Center and the University of South Florida in Tampa.

#### Early sightings

**Although MDSC-like cells have been known since the 1970s, "the association with cancer is recent,"** says Vincenzo Bronte of the Istituto Oncologico Veneto in Padua, Italy. It can be traced partly to researchers' efforts to find out why cancer vaccines weren't working.

About 10 years ago, for example, Bronte, then working with cancer immunologist Steven Rosenberg at the U.S. National Cancer Institute in Bethesda, Maryland, got a surprising result when he immunized mice with a tumor antigen and then gave a booster shot of the same antigen 6 days later. The animals' immune response was not enhanced as expected. It was suppressed instead. Bronte and his colleagues traced the

problem to an unusual group of immune cells—later called MDSCs—that were somehow taking out the CD8<sup>+</sup> T cells that would normally respond to the antigen in the vaccine.

Analysis revealed that these suppressors were immature cells from the myeloid line that produces macrophages and the dendritic cells that are needed to trigger immune responses. Their normal function, the researchers proposed, is to help put the brakes on immune responses so that they don't run out of control.

## CANCER IMMUNOLOGY

# Cancer's Bulwark Against Immune Attack: MDS Cells

First noticed in the 1970s, myeloid-derived suppressor cells appear to play a key role in sustaining tumors; new methods of overcoming them are being tested

For decades, researchers have been engaged in a frustrating effort to harness the power of the immune system to fight cancer. The approach works well enough in test tubes and experimental animals. Many types of cancer cells are studded with antigens that distinguish them from normal cells, and activated immune cells can seek out these targets and kill the cells that carry them. Yet attempts to destroy tumors by sparking similar responses in human patients, using so-called cancer vaccines and other immunotherapies, have largely ended in failure. Now, researchers may have an answer to this puzzle: A recently identified class of immune cells may help sabotage these efforts.

Within the past few years, **researchers have found that production of cells known as myeloid-derived suppressor cells (MDSCs) is markedly increased in cancer patients.** As their name suggests, MDSCs are potent suppressors of several facets of the immune system. By damping down antitumor responses, **MDSCs might contribute both**

**to the original growth of the cancers and to the failure of immunotherapies.**

**MDSCs might help explain another aspect of cancer biology as well: the apparent link between tumor growth and chronic inflammation** (*Science*, 5 November 2004, p. 966). Regulatory molecules, or cytokines, produced either by the cancer cells themselves or by other cells in the tumor environment, help trigger MDSC accumulation. And many of these cytokines also promote inflammation, **suggesting that MDSCs may be at least partly responsible for inflamma-**



**MDSC pioneers.** Vincenzo Bronte (left) and Dmitry Gabrilovich (right) were instrumental in linking MDSCs to cancer growth.

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In cancer patients, though, their long-term persistence is a problem. “There’s nothing special about these cells; they’re normal immature myeloid cells,” Gabrilovich says. But, he adds, they “are supposed to differentiate normally and not get activated and hang around in this state.”

As researchers soon learned, cancer leads to increased myeloid suppressor cells even without vaccination. In the late 1990s, Gabrilovich, in collaboration with M. Rita Young at Loyola University Chicago in Illinois, was also trying to find out why cancer vaccines are so ineffective. “We started looking at mice with tumors,” he recalls, and found that as much as 40% of the cells in the animals’ spleens—an organ that produces and stores various immune cells until they’re needed—were myeloid-derived suppressor cells. Human cancer patients, too, had three to five times more of the cells than did healthy controls. Meanwhile, the numbers of dendritic cells were decreased.

These cellular changes get more pronounced as tumors grow. **As a result, patients with advanced tumors—precisely the ones who have been in most clinical vaccine trials—have large numbers of suppressor cells that could interfere with their treatment.** “There’s no question about it; we’re going to have to deal with these cells to do immunotherapy,” says Suzanne Ostrand-Rosenberg of the University of Maryland, Baltimore County.

Researchers are beginning to explore several ways of dealing with MDSCs. One approach exploits the fact that they are developmentally immature. **To promote the differentiation of the cells, Gabrilovich and his colleagues have turned to all-trans retinoic acid (ATRA), which is already used clinically to treat people with promyelocytic leukemia.**

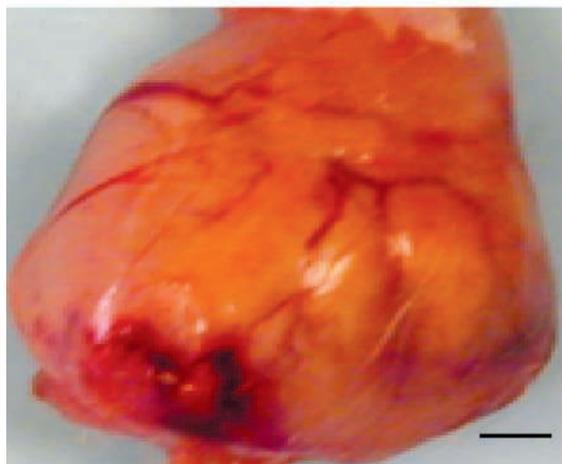
In a pilot study, the researchers gave the drug to 18 kidney cancer patients, all of whom had elevated MDSC levels. The short-term study was not designed to look for clinical improvements such as tumor shrinkage. But the immune status of the drug recipients improved; they had fewer MDSCs, more dendritic cells, and better immune responses. **The Moffitt team is now beginning a more extensive trial that will test a combination of ATRA with a cancer vaccine.** “Simply eliminating these cells won’t do,” Gabrilovich says. “You have to combine that with active immunotherapy.”

#### Versatile actors

MDSCs turn out to have many ways of blocking immune responses. They can hit both the so-called innate and adaptive branches of immunity. On the adaptive side,

they suppress antibody-producing B cells and CD4<sup>+</sup> (helper) T cells in addition to CD8<sup>+</sup> (killer) T cells.

One way they inhibit T cells is by blocking an essential activation step: the binding of antigen to the T cell receptor. About 4 years ago, Gabrilovich and his colleagues found that MDSCs release highly reactive molecules, including certain forms of oxygen and peroxynitrite. Findings from Bronte’s group



**Angiogenesis promoters.** A mouse tumor (*bottom*) exposed to VEGF-producing MDSCs has more blood vessels and grows larger than a control tumor (*top*) not exposed to the cells.

also pointed to an important role for peroxynitrite in immune suppression mediated by MDSCs. In a study published in the July 2007 issue of *Nature Medicine*, the Gabrilovich team further showed that peroxynitrite causes nitrate addition to T-cell receptors, rendering them incapable of binding antigens they would otherwise recognize.

**Other researchers are focusing on a key regulator of T cells: the amino acid arginine.** Its importance originally emerged in studies of patients who experienced serious trauma,

including surgery. **These individuals have low T-cell counts, making them very susceptible to infections, which can be fatal, particularly if they lead to a condition called sepsis.**

Researchers, including a team led by brothers Juan Ochoa, a surgeon at the University of Pittsburgh Medical Center in Pennsylvania, and Augusto Ochoa, an immunologist at Louisiana State University (LSU) Health Sciences Center in New Orleans,

**have linked this immunosuppression to low levels of arginine in the patients.** They found, for example, that in lab cultures, the amino acid is needed both for normal T-cell replication and for production of the zeta chain of the T-cell receptor. “The next question,” Juan Ochoa says, “is what was destroying arginine?”

**Further work showed that it was none other than MDSCs. These cells are loaded with the enzyme arginase, which degrades the amino acid.** About 2 years ago, Juan Ochoa and his colleagues showed that mice subjected to surgical stress produce large numbers of the cells, which proved to be potent inhibitors of T-cell activation. The researchers have also found high arginase production in cells from human trauma patients but haven’t yet pinned down the exact nature of those cells.

Meanwhile, studies of both animal models and human patients have pointed to a similar immunosuppressive role of MDSC-produced arginase in cancer. In one study about 3 years ago, the Ochoas, with LSU’s Paulo Rodriguez and colleagues, showed that arginase produced by MDSCs associated with lung cancers growing in mice impairs T-cell function in the animals by decreasing expression of the receptor zeta chain. MDSCs “interfere with the

T-cell antigen receptor so that there is no signal” to activate the cells, Augusto Ochoa says.

**The researchers also showed that treatment with an arginase inhibitor significantly slowed the growth of lung tumors in mice.** Since then, the Ochoas and others have found that **MDSCs from human cancer patients produce large amounts of arginase and nitric oxide synthase (NOS), another enzyme that degrades arginine.**

These findings suggest that treatments that raise arginine levels in T cells can alleviate the

immunosuppression occurring in trauma and cancer patients. Indeed, dietary arginine supplements have already proved useful in combating infections in trauma patients, and pre-clinical work indicates that drugs that interfere with the synthesis or function of arginase and NOS might counteract the immunosuppressive effects of MDSCs. Ivan Borrello and Paolo Serafini at Johns Hopkins University School of Medicine in Baltimore, Maryland, working with Padua's Bronte, have looked at three such **drugs—sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra)—that are much better known for their role in treating erectile dysfunction.**

As Borrello and his colleagues reported a little more than a year ago in *The Journal of Experimental Medicine*, **sildenafil in particular can decrease production of arginase and NOS by MDSCs, thereby boosting T-cell responses.** In mice with colon or mammary tumors, treatment with both the drug and the T cells primed to recognize the appropriate cancer produced much greater inhibition of tumor growth than treatment with the T cells alone. Bronte and his colleagues have found similar effects in a mouse-tumor model with an aspirin derivative called NO-aspirin.

### The inflammation connection

**Much evidence throughout the past several years has supported the idea that inflammation promotes tumor growth. Exactly how it does that isn't clear, but recent evidence implicates MDSCs.** Researchers have found that cancer cells produce a variety of proteins that either are directly inflammatory or can trigger the production of inflammatory cytokines in the tumor environment. Some foster MDSC accumulation, and, to make matters worse, MDSCs themselves have pro-inflammatory effects, thereby creating a vicious cycle that may perpetuate their own maintenance as well as tumor growth.

One early sign of an inflammatory link came from **Gabrilovich and colleagues in 1996. They found that a protein called vascular endothelial growth factor (VEGF), which is released by tumor cells, promotes the accumulation of MDSCs by blocking dendritic cell maturation.**

**VEGF helps tumors grow by stimulating angiogenesis, the formation of the new blood vessels they need. Angiogenesis is also a component of inflammation.** And about 4 years ago, two independent teams, one led by Mario Colombo and Cecilia Melani of the Istituto Nazionale per lo Studio e la Cura dei Tumori in Milan, Italy, and the other by P. Charles Lin of Vanderbilt University School of Medicine in Nashville, Tennessee, showed that **MDSCs also produce VEGF, thereby further promoting tumor growth and their own formation.**

More recent work suggests that it may be possible to break this vicious cycle. Lin and others have found that **VEGF secretion by MDSCs requires the activity of an enzyme called metalloproteinase-9.** And the Colombo team now reports that a drug that inhibits this enzyme can reduce VEGF concentrations and the number of circulating MDSCs in mice that have mammary tumors. **The drug also boosted responses to a vaccine directed against the tumor. (The results appeared in the December 2007 issue of *Cancer Research*.)**

As shown by Ostrand-Rosenberg and her colleagues, the pro-inflammatory cytokine interleukin (IL)-1 $\beta$  also stimulates MDSC production, making it another target for drugs aimed at overcoming immune suppression in cancer patients. Evidence to support this idea comes from experiments on mice lacking the

receptor through which IL-1 $\beta$  exerts its effects. As the Maryland team reported in the October issue of *Cancer Research*, mammary tumors implanted in the animals show reduced growth and metastases. "This led us to hypothesize that [MDSCs] are one of the connections between chronic inflammation and cancer," Ostrand-Rosenberg says.

Tumor cells also produce COX-2, a key enzyme in the pathway that makes inflammatory molecules such as prostaglandin E2 (PGE2). Ostrand-Rosenberg's team has found that MDSCs have receptors for the prostaglandin and that drugs that mimic its effects increase their formation while PGE2 inhibitors block it. In addition, the Ochoas and their colleagues have **found that PGE2 stimulates arginase production by the cells. And both teams have shown in mouse models that COX-2 inhibitors can slow tumor growth.** That may help explain why **individuals who take COX-2 inhibitors, which have been widely used for treating arthritis and other inflammatory conditions, seem less prone to developing cancer than people who don't take the drugs.**

Not only are MDSCs induced by inflammatory molecules, but in a situation similar to that seen with VEGF, they themselves can promote inflammation. Macrophages, which are part of the innate immune system, come in two types; M1 macrophages promote activity of killer T cells through their production of IL-12 and are thus antitumor, whereas M2 macrophages promote inflammatory responses through their production of IL-10.

In work reported earlier last year in *The Journal of Immunology*, Ostrand-Rosenberg's team found that MDSCs enhance the growth of mammary tumors in mice by interacting with M1 macrophages and converting them to the M2 type. "This sets up a strong feedback," she says, to further enhance MDSC activity. The results also indicate that the drug gemcitabine, which is already used to treat some cancers, exerts some of its effects by restoring IL-12 production by macrophages.

All in all, researchers are finding that MDSCs are extremely versatile immune suppressors and clearly a force to be reckoned with if immunotherapy is to succeed.

—JEAN MARX

