Awakening Immunity
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CANCER

Awakening Immunity

The elimination of specific stromal cells allows the immune system to suppress the growth of solid malignant tumors.

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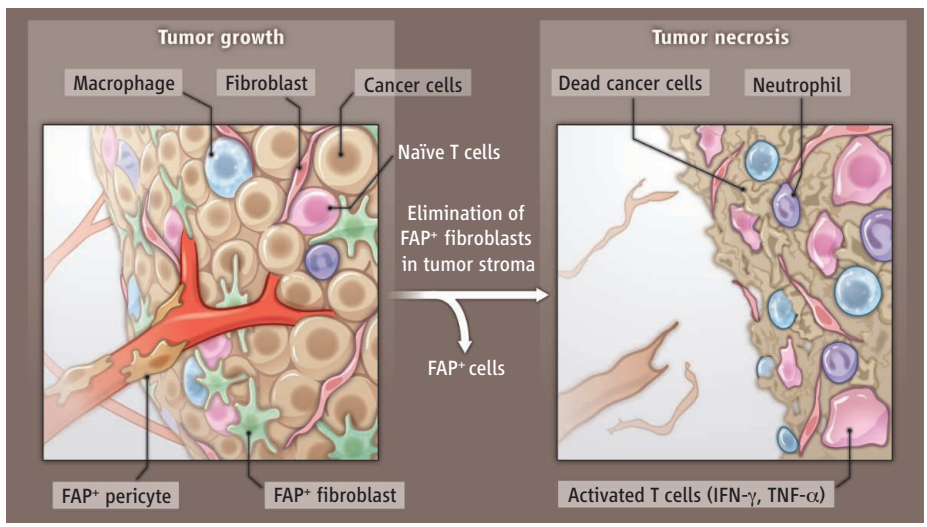
Cancer cells are embedded in stroma, the connective tissue framework of solid tumors. It consists of nonmalignant hematopoietic and mesenchymal cells, as well as extracellular matrix. Whether stromal cells have an essential role in cancer development and growth has been long debated. On page 827 of this issue, Kraman *et al*. (*1*) show that deleting a subpopulation of stromal fibroblasts arrests the growth of a solid tumor, an effect that depends on an immune response to the tumor. These results agree with other studies suggesting that immunizing against fibroblasts in tumors can unmask an immune response to cancer (*2*, *3*).

Stromal cells and cancer cells depend on each other for mutual paracrine stimulation. Stromal fibroblasts are probably required for cancer cells to survive and grow (*4*), but why does their elimination trigger an immune response to the cancer cells? A clue may come from a particular subtype of fibroblast whose removal elicits this response. Fibroblasts from malignant solid tumors show increased expression of genes that are repressed in other tissue fibroblasts (*5*), particularly the genes encoding the cytoskeletal protein α-smooth muscle actin (α-SMA) and fibroblast activation protein (FAP), a serine protease. Both proteins are also expressed on pericytes (α-SMA$^+$ and FAP$^+$), stromal cells that reside at the interface between tumor endothelium and surrounding tissue. Stromal cells expressing these markers may suppress the immune response to tumors as a consequence of producing massive amounts of stromal cell–derived factor–1 (SDF-1/CXCL12). SDF-1 attracts regulatory T cells (CD4$^+$ subtype) into the tumor (*6*). It also causes random movement of effector T cells, which interferes with T cell–tumor cell interaction and ultimately hinders tumor destruction (*7*).

Eliminating neutrophils also produces antitumor immune effects similar to those caused by eliminating FAP$^+$ stromal cells (*8*, *9*). Neutrophils release matrix metalloproteinase 9 and elastase, which enzymatically "free" stromal fibroblast progenitor cells from bone marrow and perivascular reservoirs (*10*), allowing them to follow the SDF-1 cytokine gradient into the tumor.



Tumor destruction. Removing FAP$^+$ fibroblasts and pericytes damages the blood supply and causes some cancer cells to die. The resulting damage-associated signals, together with antigens released by dying cancer cells, triggers the production of cytokines (IFN-γ and TNF-α) by cancer antigen-specific T cells in the tumor. This results in the destruction of the remaining cancer and stromal cells by the immune system.

Metalloprotease released from neutrophils also catalyzes the release and activation of latent transforming growth factor–β (TGF-β) from the extracellular matrix. TGF-β1 activates stromal fibroblasts (α-SMA$^+$ and FAP$^+$), causing them to produce immunosuppressive SDF-1. TGF-β1 also prevents the initiation of effector T cell responses. Furthermore, depending on the stimulus, neutrophils and other leukocytes can themselves produce large amounts of TGF-β1 (*11*).

Kraman *et al*. deleted FAP$^+$ stromal cells from mice bearing solid tumors that arose from injected lung cancer cells. These cancer cells were engineered to express ovalbumin, a xenoantigen capable of eliciting an immune response. The removal of FAP$^+$ fibroblasts did not alter the number or subtypes of tumor-infiltrating T cells, but did result in their activation and secretion of the pro-inflammatory cytokines interferon-γ (IFN-γ) and tumor necrosis factor–α (TNF-α) (see the figure). But in an actual tumor, what type of cancer cell antigens would be suitable for effective tumor destruction by the immune system? Kraman *et al*. emphasize the importance of unmutated self-antigens on cancer cells as effective elicitors. Although targeting by the immune system of viral antigens such as EBNA3 can eradicate large, bulky masses of lymphomas induced by Epstein Barr virus, it is not known what the critical characteristics of an antigen are that allow effector T cells to destroy large, established solid tumors or aggregates of cancer cells that have dispersed from the primary tumor to the rest of the body. EBNA3 is not expressed on normal cells and is essential for the cancer cells to remain malignant. The closest correlate to antigens on cancers that are not associated with viruses are tumor-specific mutant proteins that are essential for maintaining malignancy.

In this context, FAP as a self-antigen may be problematic because it is expressed on nonmalignant cells and its expression can be lost (*12*). Nevertheless, FAP is expressed on some fraction of stromal fibroblasts in more than 90% of patients with solid tumors, and patients with higher FAP expression have a worse clinical outcome. Thus, as Kraman *et al*. suggest, targeting FAP-expressing stroma cells for destruction could unmask the patients' own or adoptively transferred immunity. But in which clinical settings could these results influence future immunotherapy of cancer?

Human cancers when first detected usually have an average diameter of at least 1 cm and contain about 10^9 cancer cells, including thousands of diverse heritable variants resistant to drugs, radiation, and immunotherapy. Metastatic cells may already be widely dispersed. Kraman *et al*. treated relatively small tumors in mice soon after they were inoculated with

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cancer cells. Eliminating FAP$^+$ stromal fibroblasts should inhibit growth of small spontaneous tumors and thus may help eliminate clinically undetectable cancer cells that have already metastasized before excision of the primary tumor, a common cause of relapse. The caveat is that metastatic cancer cells are not necessarily in the milieu of inflammation caused by experimental cancer cell injection. Experimentally, tumors 1 cm in diameter or larger in mice are abolished by cancer-specific T cells that target not only cancer cells but also stromal cells that also present cancer cell antigens (*13*, *14*). The elimination of cancer cell variants by the immune system is presumably due to "bystander killing" that is secondary to elimination of stroma (*13*, *14*). Thus, immunotherapy treatment with both T cells that target cancer cells and an agent that targets FAP-expressing cells for destruction could increase the success of eliminating solid tumors and metastatic cells.

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PHYSICS

Antimatter Atomic Physics

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Positronium converter
Positrons
Positronium
Laser excitation
Excited positronium
Excited antihydrogen
Accelerating electric field
Antihydrogen beam
Antiproton trap

The positronium atom provides a gateway for making antihydrogen. In the proposed AEGIS experiment (*13*), Ps is to be created by bombarding a nanoporous material with positrons (Ps converter) and then laser-exciting it to a highly excited state. The excited Ps then exchanges its positron with a cold, trapped antiproton to produce highly excited antihydrogen, which is accelerated in an electric-field gradient to form a beam. ATRAP has shown the feasibility of a similar scheme. ATRAP and the former ATHENA collaboration have made antihydrogen by mixing positrons with antiprotons in a nested-well trap (*9*).

Positronium, the atom formed from an electron and a positron, is a gateway to very cold antihydrogen atoms and possibly a γ-ray laser.

Positronium (Ps), the bound state of an electron and its antiparticle, the positron, is the lightest neutral atomic species. The atomic nucleus is replaced by a positron that has only 1/1836 the mass of a proton. One way to characterize Ps atoms is to study how they scatter off other atoms and molecules, and it would be reasonable to expect Ps scattering to be some kind of coherent combination of electron and positron scattering. On page 789 of this issue, Brawley *et al*. (*1*) show experimentally that for impact energies up to 250 eV (*2*), Ps scatters as if it were just a free electron moving at the same speed. This result implies that the positron's interaction with the target is somehow "cloaked." Whether each component of the total scattering, for example, the ionization contribution, is cloaked, or whether cancellation effects are at work, remains an outstanding and substantial theoretical challenge.

Positron interactions with atoms and molecules are difficult to treat theoretically because of the high degree of correlation (*3*). Theory has to describe how the light, agile positron competes with the slow, heavy atomic nuclei for the "attention" of the electrons in the system. Thus, it was only in 1997 that the first bound state of a positron with an atom was definitely established theoretically (*4*). The existence of such bound states between a positron and a molecule has subsequently been invoked to explain extremely high positron annihilation rates in certain molecular gases (*5*). The proposed mechanism involves temporary capture of the positron by a molecule that is raised to an excited vibrational state. This state traps the positron for a sufficient length of time to markedly enhance its chance of annihilation by a molecular electron. Positron annihilation is the basis of medical positron emission tomography (PET) scanning. Because a positron will eventually be annihilated by an electron, even stable bound states have a short lifetime, on the order of 1 ns (10^{-9} s). Experimental observation of bound states is problematic. For example, it is only recently that the Ps_2 bound state (the analog of H_2), predicted in 1947, has been observed (*6*).

The lifetime of bound systems depends on the alignment of the electron and positron spins. In Ps, there are two possible spin combinations, singlet and triplet, called para-Ps and ortho-Ps. The annihilation of an electron and positron in the lowest energy state of para-Ps predominantly forms two very high energy photons (γ-rays of 511 keV each) with a lifetime of 0.125 ns. Ortho-Ps is prevented by conservation laws from annihilating directly into two photons, and so annihilates predominately into three photons with a lifetime of 142 ns. Only the ortho form of Ps is sufficiently long lived to be used in the beam experiments of Brawley *et al*.

These properties are relevant to an ambitious project to make a Bose-Einstein condensate of Ps (*7*), which would be the first example of a matter-antimatter condensate The problem is to create a sufficiently dense gas of ortho-Ps in a microscopic cavity in a suitable material such as silica. The difficulty is that Ps-Ps collisions could convert ortho-Ps into para-Ps, which would then rapidly undergo destruction. However, if the electron and positron spins in all of the Ps atoms can be aligned in the same direction, this decay mechanism is not available. Recently, this type of spin alignment has been shown to be experimentally possible (*8*). If a condensate can

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