Tireless surveillance by exhausted T cells

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T cell exhaustion is an evocative concept that results in attenuated function in the face of chronic antigen exposure and is critical to avoid immunopathology. However, tumors often exploit this dampened T cell function to escape the antitumor immune response. In this issue of the *JCI*, You et al. investigated a different aspect of T cell exhaustion in the setting of tumor immunity by characterizing the capacity of T cells for tireless migration. The dynamic nature of normal T cells was first made famous by intravital microscopy studies in explanted tissues. You et al. used a similar imaging strategy with reanimated human tumors, in which exhausted T cells displayed an enhanced capacity for intratumoral motility. These results suggest that exhausted T cells may be able to teach T cell engineers lessons about navigating within the tumor microenvironment.

Tumor immune evasion

T cells start their lives as nomads of the body (1). They use the blood like a high-speed rail system to move between immunological cities, the lymphoid tissues, where T cells mix with antigen-presenting DCs to match antigen receptors with antigens. Labyrinthine stromal cell networks use chemokines to signal T cells and DCs to engage in a frenetic migration that maximizes the chances for T cells with a single receptor to find potential matches with peptide-MHC complexes presented on the DCs (2). When T cells encounter sufficient activating peptide–MHC along with evidence of innate immune activation, the T cells decelerate and dwell longer with particular DCs, undergo rapid expansion, and differentiate into effector cells that set out from the lymphoid tissue to sites of infection or cancer (3). In infection, the renewed surveillance enables a search-and-destroy mission, often with violent tissue damage. If the infection can't be cleared efficiently, the chronic presence of antigen and other signals triggers a program to attenuate the T cell response, one aspect of which is the conversion of effector T cells into functionally attenuated exhausted T cells (4). Tumor immune evasion can take advantage of this host protective program, enabling tumor growth despite an immune response. Since the exhaustion program can eventually control and even clear viruses over a period of months, it is not considered a surrender. Many would-be tumors are likely kept in check or eliminated by antitumor immune responses, and checkpoint therapies can reactivate immune responses from a substrate of intratumoral T cell exhaustion (5). But what does this program look like in the tumor? In this issue of the *JCI*, You et al. from the Krummel laboratory used mouse models and imaging in human tumor explants to provide insight into the lifestyle of exhausted T cells (6).

Imaging methods for human tissue explants

Our understanding of the in situ dynamics of immune responses has been driven by laser scanning microscopy (7). Conditions were established on the basis of technology for imaging live brain tissue slices for imaging lymphoid tissue explants, which gave results that were effectively identical to those of much more technically difficult in vivo imaging in lymph nodes (8). These imaging techniques opened the door for the development of imaging methods for human tissue explants. The Donnadieu research group overlayed tumor slices with fluorescently labeled human T cells, which infiltrated the slices and displayed the characteristic interstitial migration observed in mouse studies (9). A few studies have injected fluorescent antibodies to label and track immune cell populations in mouse tissues (10). This technique suggests a strategy for labeling endogenous immune cell populations within human tumor slices. Krummel's team established parallel mouse and human models to validate imaging of endogenous populations in tumor slices. In the mouse studies, T cells were labeled with genetically encoded fluorescent proteins. Two differentially labeled T cell populations were transferred into tumor-bearing mice, and the tumor was either directly imaged in the live mouse or removed and imaged ex vivo to investigate T cells that had entered the tumor within four days (effectors) or 14 days (exhausted). Exhausted T cells displayed fast migration in both the in vivo and explant tumor slice models in the mouse, generating a green light for the human explant studies. The human explant approach was based on non–function-blocking monoclonal antibodies against CD8, CD14, or MHC class II, and EpCAM to track T cells, antigen-presenting cells, and tumor cells, respectively. These antibodies were directly conjugated with quantum dots to provide bright, photostable signals for two-photon laser scanning microscopy. The use of the bivalent IgG with intact Fc in the study by You et al. does pose some risks of perturbing normal behavior, but hopefully, the success of this initial effort will encourage further commercial development of small, monova-

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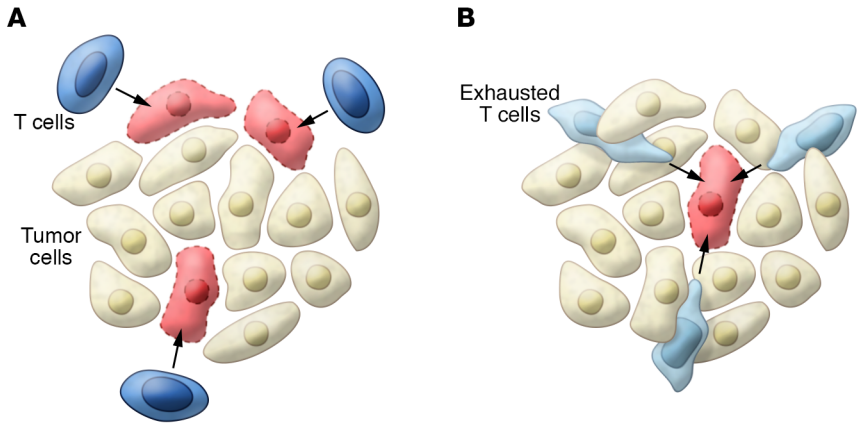

Figure 1. Cumulative damage model for the motile exhausted T cell's muted attack. (**A**) Newly recruited effector T cells (blue) engage targets at the interface between the tumor (yellow) and healthy surrounding tissues, leading to effective killing of superficial tumor cells (red). (**B**) Later in the antitumor response, exhausted T cells (light blue) become highly motile. The highly motile exhausted T cells may penetrate the tumor better, but are also weaker effectors, so they need to inflict cumulative damage through many encounters to kill tumor cells (red). The mobility program of exhausted T cells could be useful in immunotherapy applications that improve T cell numbers and/or potency to increase deep tumor killing.

lent probes, such as nanobodies, with the appropriate fluorescence dyes to support this area of research. Currently, the need to custom-generate such reagents and the loss of signal intensity due to weaker binding remain technical challenges. Nonetheless, it would be exciting if the monovalent probes labeled with the brightest organic dyes (quantum dots don't always fit in synapses) could track functionally relevant molecules. For example, molecules involved in T cell–APC interaction, such as CD2 and LFA-1, could potentially be followed by monovalent antibody fragments targeting non–function-blocking epitopes (11). Despite the mild caveats, the technical platform established by Krummel and colleagues provided exciting results.

Classifying T cells within tumors

You and colleagues made compelling observations in their current work (6). They sampled multiple regions of each tumor slice and found that the speed of T cell movement was inversely correlated with the density of cancer cells, which is similar to the results obtained by Donnadieu and colleagues regarding the role of extracellular matrix in preventing T cell migration into tumor cell islets (9, 12). The researchers were nonetheless able to classify the T cells within the tumors as motile or immotile. In tumors with motile T cells, the T cells had higher expression of exhaustion markers than did tumors with immotile T cells. In the parallel mouse model, You et al. noted that motility was proportional to programmed cell death 1 (PD-1) expression, as determined by tracking endogenous T cells labeled with anti–PD-1 antibody, again, demonstrating the power of the simple antibody-based labeling approach to generate quantitative information in situ, even when targeting a functional protein like PD-1. In addition, the authors identified a motility-associated gene expression signature in exhausted T cells. The identification of this signature is a particularly exciting result, as it suggests that exhausted T cells have adaptations allowing them to navigate in tumor microenvironments that may challenge T cell dynamics, as noted in earlier intravital microscopy studies (13). A goal of T cell immunotherapy might be to boost effector programs while further promoting the tireless motility program of exhausted T cells (12). T cells can initiate killing programs quickly (14), and additive damage-based T cell killing might enable an effective swarming attack (15–17).

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