acting. Because they act on the immune system, they might have unexpected, unwanted side effects that will need to be investigated. Furthermore, although the results by Xu et al.\textsuperscript{1} are impressive, the degree of the effect on pain behavior in the animals was moderate—the pain seemed to be attenuated but not completely abolished. Other analgesic drugs, which have had stronger effects than the resolvins in the animal models, have failed in clinical trials because their impact was not sufficiently different than that of a placebo.\textsuperscript{10,11} Thus, the results of clinical trials with resolvins will be eagerly awaited.

Given the results presented by Xu et al.\textsuperscript{1}, acute complex regional pain syndrome, which is characterized by localized exaggerated post-traumatic inflammation and peripheral and central nociceptive sensitization,\textsuperscript{12,13} might be a good candidate disease to be treated by resolvins. If resolvins turn out to be efficacious in the acute phase of complex regional pain syndromes stage, they will help prevent long-term suffering and loss of function in patients.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.


T cell receptors and cancer: gaines pain

Malcolm Brenner

T lymphocytes engineered to produce T cell receptors specific for tumor antigens lead to an antitumor immune response. New findings draw attention to a potentially deadly problem with this strategy. The transgenic T cell receptors can shuffle components with native receptors to produce hybrid molecules with specificity against self antigens (pages 565–570).

The phrase “war on cancer” has been widely derided, but war imagery helps illustrate how the treatment of cancer has been hampered by the lack of agents that specifically target “insurgent” cells and cause minimum collateral damage. Researchers have thus turned to the immune system, with its excellent capacity to target foreign invaders, to gain greater selectivity against tumors and hence fewer adverse effects compared to chemotherapy and radiation.

Monoclonal antibodies are already showing their value in cancer treatment, but immune cells, such as T lymphocytes, should be even more accurate and effective in destroying cancer. T lymphocytes are highly specific for particular antigens and, unlike monoclonal antibodies, they are capable of actively cruising through tissues to seek out cancer cells. Investigators have been directing T lymphocytes specifically toward tumor targets by introducing transgenes encoding T cell receptors (TCRs) that recognize proteins expressed either uniquely or in much higher amounts on the surface of tumor cells compared to normal tissues.

But as Bendle et al.\textsuperscript{1} now show, these reprogrammed cells are potential double agents. In this issue of *Nature Medicine*, the researchers show that these cells, once modified, can shuffle the components of their native and transgenic TCRs.\textsuperscript{1} As a result, the cells lose their cancer targeting activity and gain specificity against normal host tissues. The consequences are lethal.

Cytotoxic T lymphocytes (CTLs) are a class of T lymphocytes that bind particular antigens presented by the major histocompatibility complex (MHC) on cells and then proceed to destroy those cells. CTLs taken from individuals with cancer and expanded \textit{ex vivo} can effectively treat a minority of human tumors when they are reintroduced into the same patients.\textsuperscript{2–4} But most tumor-associated or tumor-specific antigens are not unique to tumors. They are instead self proteins to which the immune system was rendered unresponsive during development.\textsuperscript{5} Hence, tumor antigen–specific CTLs isolated from people with cancer before \textit{ex vivo} expansion and re-infusion typically have only low-affinity antigen–specific TCRs, limiting their cytotoxic activity against tumor cells.

Investigators have overcome this limitation by using gene transfer to express in human CTLs transgenic TCRs that have high affinity for particular tumor antigens,\textsuperscript{2} allowing rapid production of large numbers of tumor–antigen–specific CTLs. Morgan et al.\textsuperscript{3} used the approach to genetically modify T cells to produce TCRs specific for MART-1, an antigen present on melanoma cells, and then returned the cells to patients with melanoma. They reported regression of metastatic lesions and prolonged persistence of CTLs in two patients.\textsuperscript{6}

One major constraint of such TCR gene transfer, however, is the development of cross-paired hybrid TCRs. TCRs consist of two chains, designated \(\alpha\) and \(\beta\). A single CTL expresses thousands of TCRs with the same \(\alpha\) and \(\beta\) chains. When another TCR is introduced by gene transfer, the native \(\alpha\) or \(\beta\) chains can cross-pair with the reciprocal transgenic chains to produce a new, hybrid TCR. Although the existence of these cross-paired receptors has long been appreciated, most investigators assumed they would simply lose antigen specificity, reducing the effectiveness of the genetically modified cells (Fig. 1). Bendle et al.\textsuperscript{1} show that the hybrid receptors are also capable of gaining new functions, producing a lethal variant of graft–versus–host disease (Fig. 1).

Bendle et al.\textsuperscript{1} introduced a transgenic \(\alpha\) and \(\beta\) TCR directed against ovalbumin (OVA-TCR)—an antigen absent on normal mouse cells—into the T cells of mice and then injected these cells into mice that had been lymphodepleted by low-dose irradiation. They also gave the mice the T cell growth factor interleukin–2. Fourteen days after the

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transgenic T cells were infused into the mice, the authors noted that the mice had developed severe cachexia, bone marrow failure, lymphopenia and a high frequency of colitis and pancreatitis — some of the features of graft-versus-host disease 1.

The authors assiduously showed that all of these disease manifestations could be attributed to the presence of the infused TCR-transgenic T cells, which had gained specificity for new, unidentified targets 1. This new and unwanted specificity of the transgenic receptors seemed to be due to the formation of hybrid receptors with endogenous TCR molecules, as the introduction of even a single α or β transgenic receptor chain was sufficient to produce the effect.

The observed effects of the transgenic T cells on mice were associated with high levels of interferon-γ production by these cells, as OVA-TCR–transgenic T cells from interferon-γ–deficient mice did not produce any disorders. The authors also showed that the OVA-TCR is not uniquely promiscuous in its ability to form potentially lethal cross-pairings with endogenous TCR chains, as they obtained similar results with four out of five other transgenic TCRs, albeit at lower frequency and with reactivity against an apparently more restricted array of antigenic targets than the OVA-TCR 1.

The autoreactivity observed after infusion with TCR-OVA–expressing T cells could perhaps have been predicted, as a substantial percentage of peripheral blood T cell TCRs react with allelicogenic major and minor histocompatibility antigens, which are polymorphic from individual to individual. Whereas T cells that react with autologous histocompatibility antigens are normally deleted or anergized during T cell development, the introduction of large numbers of cells with an entirely new assortment of TCRs by cross-pairing is likely to include many that are self reactive. Indeed, Bender et al. 1 show that an oligonodal or monoclonal population of OVA-TCR–transgenic cells, which have a much smaller assortment of such cross-paired receptors than polyclonal OVA-TCR cells, do not produce the graft-versus-host–like syndrome.

How clinically relevant are these observations? Mice are not homunculi, and, to date, autoreactivity has not been observed in human studies where patients are given transgenic CTLs, even when the patients, like the mice in the current study, are lymphodepleted and receive interleukin-2 (ref. 6). Bender et al. 1 argue that the lack of severe autoreactivity in human subjects may simply be attributable to a fortunate choice of transgenic TCR. It may also reflect the longer and greater ex vivo expansion of transgenic T cells required for human studies, as such manipulations reduce subsequent in vivo growth.

Irrespective of the ultimate frequency or severity of this problem in humans, it is clearly essential to develop approaches that prevent cross-pairing from occurring and produce defined and predictable TCR transgene products suitable for clinical studies. Several such approaches have already been described and shown to work in preclinical studies 5–7. But given the concerns about transgenic αβ TCRs, it may also be worthwhile to explore further the alternative approach of transferring synthetic chimeric TCRs into T cells. These chimeric TCRs, called chimeric antigen receptors (CARs), are most commonly generated by joining the light and heavy chain variable regions of a monoclonal antibody, expressed as a single-chain Fv (scFv) molecule, with other TCR components, such as the ζ chain and the signaling domains of T cell co-stimulatory molecules such as CD28. CARs only recognize surface antigens. They do not recognize the MHC-associated peptides of internal tumor-associated antigens that bind the classical TCR. Future identification of CARs with high affinity for tumor-derived peptides in their associated MHC binding groove may overcome this obstacle 8.

Ultimately, however, the effect of a weapon depends on how it is wielded, and it may become essential to be able to destroy modified T lymphocytes that misbehave. One such strategy is to transfer along with the TCR the gene encoding the herpes simplex viral thymidine kinase, which phosphorylates the prodruk ganciclovir to inhibit DNA synthesis 9. This approach is already in late-phase clinical trials, and newer approaches—for example, using an inducible component of the endogenous caspase pathway, which induces rapid T cell apoptosis on exposure to the triggering drug—are showing preclinical and early clinical promise 10. Their introduction may eventually provide cellular agents for cancer therapy that are truly targeted, effective and safe.

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