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See related article, “Carotid Endarterectomy Benefits Patients with CKD and Symptomatic High-Grade Stenosis,” on pages 145–152.

## Protection from Cancer in Kidney Transplant Patients by $\gamma\delta$ T Cells: Role of CMV Infection?

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In this issue of *JASN*, Couzi *et al.*<sup>1</sup> describe a longitudinal case-control study of kidney transplant recipients who developed cancer 2 to 6 years after transplantation. The median percentage of  $\gamma\delta$  T cells was significantly lower in patients who developed cancer but only in those who had a pre- or posttransplantation cytomegalovirus (CMV) infection.

$\gamma\delta$  T cells reside among the epithelia of various organs, particularly in lung, skin, intestine, and tongue, where they serve as a first-line defense against invading viruses, bacteria, and fungi. Activated early, during the interphase between innate and adaptive immunity,  $\gamma\delta$  T cells attract innate effector cells and help polarize the adaptive immune response.<sup>2</sup> When they recognize cells undergoing environmental stress or infection, they produce cytokines (IFN- $\gamma$ , TNF- $\alpha$ , IL-1, and IL-17) that activate the adaptive immune system.<sup>3</sup> The site of accumulation and the stimuli that activate  $\gamma\delta$  T cells depend on their receptor V $\delta$  regions. For example, V $\delta$ 2  $\gamma\delta$  T cells recognize nonpeptidic phosphorylated metabolites of isoprenoid biosynthesis expressed by mycobacteria, whereas V $\delta$ 1  $\gamma\delta$  T cells are activated by MIC-A, MIC-B, and UL-16-binding proteins and thus respond to NKG2D ligands through an MHC-independent mechanism that does not require recognition of specific antigens.<sup>4</sup> This makes them particularly interesting in tumor surveillance, because tumor cells often downregulate MHC class I molecules.

$\gamma\delta$  T cells are more numerous after CMV infection, and an increase in V $\delta$ 2<sup>−</sup>  $\gamma\delta$  T cells associate with clearance and control of infection.<sup>5–7</sup> CMV-infected cells are targets for V $\delta$ 2<sup>−</sup>  $\gamma\delta$  T cells,<sup>5–7</sup> and infection induces a memory response.<sup>8</sup> The factors on CMV-infected cells that activate V $\delta$ 2<sup>−</sup>  $\gamma\delta$  T cells are unknown; however, the expansion of this population is puzzling because CMV downregulates factors that may be involved in their activation, such as MIC-A, MIC-B, and UL-16-binding proteins, as well as MHC class I molecules.

CMV is a common virus in the population, and 50 to 90% of adults have been previously infected with CMV and carry latent virus. The primary infection is generally asymptomatic or causes mild symptoms; however, patients with a suppressed immune system, such as transplant recipients and patients with AIDS, develop clinical CMV infections that may be life-threatening. The virus remains dormant, particularly in blood cells of the myeloid lineage, and can reactivate during inflammation.<sup>9</sup> CMV may also reactivate in patients who have cancer and are immunosuppressed as a

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result of chemotherapy,  $\gamma$  radiation, or cancer-related immunosuppression.

A role for CMV in cancer was first proposed in the early 1970s, when increased serum titers of CMV antibodies were found in patients with cervical carcinomas.<sup>9,10</sup> Shortly thereafter, CMV was observed in prostate cancer, and a viral strain that formed new tumors in nude mice was isolated.<sup>10</sup> This experiment was difficult to repeat, and at the time it was concluded that CMV most likely was not oncogenic.

In 2000, Cobbs *et al.*<sup>11</sup> revisited the potential role of CMV in clinical cancer. Using high-sensitivity staining protocols, they found CMV in 100% of malignant glioblastomas and >90% of colon, breast, and prostate cancers.<sup>9</sup> Importantly, nearby normal cells were consistently CMV negative. Other groups, including our own, confirmed these findings in patients with malignant glioblastoma but only when optimized techniques were used for CMV detection. Viral DNA, mRNA, and proteins have been found in tumors, but infectious virus has not been isolated. Thus, CMV does not behave like a normal viral infection in these tumors—an observation that perhaps is key to understanding its biologic role in cancer.

What is the significance of CMV in the tumor? It has been proposed that CMV reactivation may be an epiphenomenon in certain cancers. In malignant glioblastomas, the level of CMV in the tumor is a strong prognostic factor for patient survival. Patients with low-grade tumor CMV infection lived three times as long as those with high-grade infection (42 *versus* 14 mo;  $P = 0.0008$ ), suggesting a causative role of CMV in these tumors (unpublished observations). In a clinical trial, we are evaluating the efficacy of antiviral drugs against CMV in patients with malignant glioblastoma.

If the virus infects tumor cells, then how does it affect tumor progression, and why is the immune system so poor at recognizing these tumors? One reason is that CMV proteins provide sophisticated strategies that enable the virus to avoid immune recognition and turn infected cells into efficient viral factories. The CMV genome has 252 known open reading frames, approximately 170 of which are estimated to encode viral proteins,<sup>9</sup> but only approximately 50 proteins are essential for viral replication. Thus, most CMV proteins are devoted to helping the virus coexist with its host. Regulatory CMV proteins and noncoding RNAs can affect tumor cells by controlling cell-cycle progression; inducing proliferation and chromosomal instability; causing epigenetic modifications; and increasing survival, angiogenesis, invasiveness, and telomerase activity—all of which affect tumor progression.<sup>10</sup> These mechanisms are believed to be oncomodulatory rather than oncogenic functions.

CMV also induces inflammatory processes that may be involved in cancer initiation and progression. CMV induces expression of cyclooxygenase 2 (COX-2) and 5-lipoxygenase, two key enzymes in the biosynthesis of inflammatory mediators from arachidonic acid.<sup>9</sup> Both are expressed in

many tumors, and intervention studies demonstrate some benefit of COX-2 inhibitors in cancer prevention. A chemokine receptor homologue produced by CMV, US28, activates NF- $\kappa$ B, COX-2 expression, and vascular endothelial growth factor production in 3T3 fibroblasts, which result in malignant transformation and tumor development in nude mice.<sup>12</sup> COX-2 inhibitors prevent this tumor development, which suggests a unique link among CMV infection, inflammation, and tumor development.

Little is known about the specific response against CMV in tumors. Prins *et al.*<sup>13</sup> demonstrated that dendritic cell vaccination with a tumor lysate from malignant glioblastoma induced a marked immunologic reactivity against CMV-pp65. This observation suggests CMV proteins are present in the tumor and trigger CMV-specific T cell reactivity; however, CMV has numerous other strategies for avoiding immune recognition and inducing clinical immunosuppression, and specific immunologic reactivity may be dampened in patients with CMV-infected tumors.

Increasing evidence now suggests that  $\gamma\delta$  T cells confer this antitumor activity. These cells infiltrate different tumors, including breast cancers, renal cell carcinomas, seminomas, and lung cancers,<sup>14</sup> and experimental studies suggest that both  $V\delta 2^-$  and  $V\delta 2^+$   $\gamma\delta$  T cells kill different tumor cell lines from a variety of malignancies *in vitro*. Mice lacking  $\gamma\delta$  T cells are prone to develop tumors induced by cutaneous carcinogens.<sup>15</sup> The study by Couzi *et al.*<sup>1</sup> provides insights into the potential role of CMV in cancer and highlights differences in immunologic reactivity against tumor cells or the virus that determines the number of circulating  $\gamma\delta$  T cells in infected individuals. Thus, a reduced number of such cells may identify patients who are at increased risk for developing cancer after transplantation.

The report by Couzi *et al.*<sup>1</sup> also raises some interesting questions. Does the increased number of  $V\delta 2^-$   $\gamma\delta$  T cells demonstrate a direct relationship between CMV infection and immune control of certain tumors? If so, then does this relationship extend to other forms of cancer, and can a simple blood test identify patients who are at high risk for developing cancer? Can specific  $\gamma\delta$  T cells be used for immunotherapy in cancer? In patients with malignant glioblastomas, the total number of  $\gamma\delta$  T cells is lower than in control subjects,<sup>14</sup> and recent reports demonstrated a high frequency of CMV in these tumors, which is associated with immunosuppression. Thus,  $\gamma\delta$  T cell therapies merit further investigation as a potential treatment option for these patients.

In three cases, Couzi *et al.*<sup>1</sup> examined kidney tumors for CMV by PCR but found no signs of virus. Many laboratories have failed to detect the virus by nonoptimized techniques. Thus, it is still possible those CMV proteins were present in the kidney tumors and that the increased number in  $V\delta 2^-$   $\gamma\delta$  T cells reflects a response to numbers of CMV-infected cells in the tumors. Alternatively,  $V\delta 2^-$   $\gamma\delta$  T cells may expand due to a systemic CMV infection, as in kidney transplant recipients and patients who boost the number of these cells may combat

cancer better. It is also possible that V $\delta$ 2<sup>-</sup>  $\gamma$  $\delta$  T cells are boosted only in response to tumor cells and that patients who mount a robust response may combat cancer more efficiently.

Further understanding of the expansion and function of  $\gamma$  $\delta$  T cells may bring us closer to understanding the role of CMV in cancer and reveal whether immunotherapies using  $\gamma$  $\delta$  T cells may eventually prove useful for treating certain cancers. Meanwhile, it will be important to determine whether V $\delta$ 2<sup>-</sup>  $\gamma$  $\delta$  T cells serve as a biomarker for risk for cancer in CMV-infected individuals.

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## DISCLOSURES

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See related article, "Cytomegalovirus-Induced  $\gamma$  $\delta$  T Cells Associate with Reduced Cancer Risk after Kidney Transplantation," on pages 181–188.