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Roles for $CD8^+$ T Cells and IL-10 in the Resolution of Paclitaxel-Induced Neuropathic Pain

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Review of Krukowski et al.

Peripheral neuropathy induced by antineoplastic drugs is one of the most common neurological complications of cancer treatment, affecting up to 80% of patients. Paclitaxel, a widely used anticancer drug for solid tumors, such as breast cancer, frequently produces dose-limiting peripheral neurotoxicity characterized by paresthesia, dysesthesia, and neuropathic pain (De Iuliis et al., 2015). Unfortunately, there are no clinically validated treatments to counteract this neuropathy, partly because its pathophysiological mechanisms are only partially known. But animal models of paclitaxel-induced neuropathic pain are helping to elucidate its pathogenesis and make it possible to test experimental treatments (Hopkins et al., 2016).

Increasing attention is being focused on non-neuronal mechanisms that may amplify or resolve chronic pain, and cells traditionally believed to act as coordinators of the inflammatory response are now recognized as modulators of pain signaling. In response to nerve injury, non-neuronal cells, such as leukocytes and glial cells, release neuromodulatory substances that can act on sensory neurons to either promote or reduce pain (Ji et al., 2016). Paclitaxel-induced neuropathic pain is associated with a neuroinflammatory process involving activation of glial cells in the spinal cord and satellite cells along with macrophage infiltration into the DRG. Together, these changes led to the production of pronociceptive mediators, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (Sisignano et al., 2014), and these neuroinflammatory processes may contribute to the pain induced by this anticancer drug.

Although symptoms of paclitaxel-induced neuropathic pain sometimes cease when treatment is stopped, they sometimes become chronic (Boyette-Davis et al., 2015). Similarly, preclinical models have shown that neuropathic pain-like behaviors after paclitaxel exposure can resolve (Nieto et al., 2014) or persist for several weeks or even months after treatment stops (Flatters and Bennett, 2006; Ledeboer et al., 2007). The mechanisms that resolve chemotherapy-induced pain have remained unclear. In an article published recently in *The Journal of Neuroscience*, Krukowski et al. (2016) identified a novel mechanism that contributes to the termination of paclitaxel-induced neuropathic pain.

T cells (T lymphocytes) are essential components of adaptive immunity involved in pain pathology (Ji et al., 2016). They represent a heterogeneous group of immune cells that exhibit various phenotypes distinguished by surface markers. All T cells express the CD3 marker and are divided into helper T cells ($CD4^+$) and cytotoxic T cells ($CD8^+$), with further subdivisions based on their cytokine profiles (Broere et al., 2011). To investigate the role of T cells in paclitaxel-induced neuropathic pain, Krukowski et al. (2016) used $Rag1^{-/-}$ mice, which are deficient in mature T cells, and transferred specific subtypes of T cells intravenously before paclitaxel administration.

The study by Krukowski et al. (2016) shows that T cells are essential for the amelioration of paclitaxel-induced neuropathic pain. The authors administered low doses of systemic paclitaxel to mice, which developed transient mechanical allodynia that started to resolve after 7 d, coinciding with a significant increase in the number of T cells ($CD3^+$) in the lumbar DRGs. Krukowski et al. (2016) administered paclitaxel to $Rag1^{-/-}$ mice and found that these mutant mice developed mechanical allodynia with an onset and severity similar to that seen in wild-type (WT) mice, but which continued for a longer period. When the authors transferred $CD3^+$ T cells intravenously to $Rag1^{-/-}$ mice before paclitaxel treatment, however, the resulting allodynia resolved by the same time as in WT mice.

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In contrast to these findings, two other studies that explored neuropathic pain-like hypersensitivity in Rag1$^{-/-}$ mice reported a significant reduction in mechanical (Costigan et al., 2009) and thermal (Kleinschnitz et al., 2006) hypersensitivity, which resolved over the same period as in WT mice. However, the neuropathic pain models used in the three studies have different etiologies (mechanically induced vs chemically induced nerve injury); therefore, the different results may be explained by differences in the pathophysiology of neuropathic pain in these models.

Interestingly, Krukowski et al. (2016) also identified the T-cell subset responsible for recovery from paclitaxel-induced mechanical allodynia, demonstrating normal resolution in Rag1$^{-/-}$ mice that received CD8$^+$ T cells intravenously. In contrast, mechanical allodynia persisted in Rag1$^{-/-}$ mice that received CD4$^+$ T-cell transfers. A question that these findings raise is whether CD8$^+$ T cells are the only immune cells involved in this process. Rag1$^{-/-}$ mice are also deficient in mature B cells; and although the role of these immune cells in neuropathic pain is unclear (Dawes et al., 2013), an increase in B cells in the DRG of WT mice has been reported in association with paclitaxel-induced neuropathic pain (Liu et al., 2014). However, Krukowski et al. (2016) did not investigate the effects of B cells transferred to Rag1$^{-/-}$ mice on paclitaxel-induced neuropathic pain to determine whether these lymphocytes play a hypothetical role.

In contrast to the conclusion that CD8$^+$ T cells play a key role in the resolution of paclitaxel-induced neuropathic pain, a previous report by Liu et al. (2014) found that the intrathecal administration of CD8$^+$ neutralizing antibody reversed paclitaxel-induced mechanical allodynia, whereas the adoptive intrathecal transfer of CD8$^+$ T cells exacerbated it. Therefore, different routes of administration seem to be connected with different roles of CD8$^+$ T cells. One possible explanation is that Krukowski et al. (2016) administered CD8$^+$ T cells before paclitaxel treatment, whereas Liu et al. (2014) administered the cells after treatment. In addition, Krukowski et al. (2016) transferred CD8$^+$ T cells to Rag1$^{-/-}$ mice, but these mutant mice were still deficient in CD4$^+$ T and B cells. Because both types of lymphocyte release a variety of cytokines that may modulate CD8$^+$ T-cell responses (Broere et al., 2011; Bao and Cao, 2014), the lack of CD4$^+$ T and B cells in Rag1$^{-/-}$ mice may be one of the reasons for these contradictory results.

Krukowski et al. (2016) also explored the contribution of the anti-inflammatory cytokine IL-10 to the resolution of paclitaxel-induced pain because CD8$^+$ T cells exert their regulatory effects in part through the production of this cytokine. IL-10 is a powerful anti-inflammatory cytokine with antinociceptive properties. Previous studies of intrathecal IL-10 gene therapy in animal models found that IL-10 suppressed neuropathic pain symptoms, including paclitaxel-induced neuropathy (Milligan et al., 2006; Ledeboer et al., 2007). In agreement with these reports, the study by Krukowski et al. (2016) demonstrated that the inhibition of endogenous IL-10 signaling by IL-10 knock-out (KO) mice or by the intrathecal injection of anti-IL-10-neutralizing antibody in WT mice delayed recovery from paclitaxel-induced allodynia. In addition, the intrathecal injection of anti-IL-10 antibody in Rag1$^{-/-}$ mice reconstituted with CD8$^+$ T cells also delayed recovery from paclitaxel-induced allodynia, in comparison with Rag1$^{-/-}$ mice with CD8$^+$ T cells treated with control IgG antibodies, suggesting that recovery is dependent on spinal IL-10 and mediated by CD8$^+$ T cells. However, Rag1$^{-/-}$ mice reconstituted with CD8$^+$ T cells from IL-10 KO mice (i.e., CD8$^+$ T cells unable to produce IL-10), displayed a resolution of paclitaxel-induced allodynia similar to that of Rag1$^{-/-}$ mice reconstituted with CD8$^+$ T cells from WT mice. This latter finding indicated that CD8$^+$ T cells were not the source of the spinal IL-10 needed for the resolution of mechanical allodynia. In support of this conclusion, the authors also showed a significant increase in IL-10 mRNA in the spinal cord, but not in the DRG, whereas T-cell infiltration/proliferation was found only in the DRG, but not in the spinal cord, after paclitaxel treatment. Together, these findings suggested that CD8$^+$ T cells in DRGs may modulate DRG neurons, which may in turn lead to processes that trigger the increase in IL-10 in the spinal cord.

In the CNS, microglia can assume an anti-inflammatory phenotype characterized by the release of anti-inflammatory mediators, including IL-10 (Cherry et al., 2014). Therefore, microglia may be the source of increased IL-10 in the spinal cord after paclitaxel administration, as reported by Krukowski et al. (2016). Although the role of microglia in paclitaxel-induced neuropathy is controversial, several laboratories have reported microgliosis in the mouse and rat spinal cord (Ledeboer et al., 2007; Peters et al., 2007; Burgos et al., 2012; Pevida et al., 2013) associated with paclitaxel-induced pain, even at a cumulative dose (Burgos et al., 2012) similar to that used by Krukowski et al. (2016). The functional phenotypes of microglia (proinflammatory and anti-inflammatory) in paclitaxel-induced neuropathic pain are unexplored, however. Further research is needed to identify which phenotypes are present and the endogenous signals that trigger these phenotypic changes.

In conclusion, the results reported by Krukowski et al. (2016) contribute significantly to our understanding of the mechanisms that underlie the resolution of paclitaxel-induced neuropathic pain. Preventive therapies would be particularly useful in the context of chemotherapy-induced neuropathy, where the moment of onset of the insult is known in advance. This study opens new avenues for expanding our knowledge of the mechanisms that orchestrate the chronicity of neuropathic pain, and may lead to novel interventions based on IL-10 therapy to prevent and/or treat this process. Nevertheless, the exact mechanisms of CD8$^+$ T cell and IL-10 signaling in this process remain unclear, and further research is needed to elucidate the possible role of glial cells and other antinociceptive mediators.

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