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LETTER TO THE EDITOR

Stem Cell Therapy Versus T Lymphocytes: Friend or Foe?

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DEAR EDITOR:

Given the recent findings that transplanted therapeutic stem cells may die within days of implantation, soluble factors are now thought to play a key role in the outcome of stem cell therapies [1, 2]. This brings focus on pivotal immune regulatory switches within the host and is especially relevant where the source of therapeutic stem cell influences therapeutic efficacy. By comparing human bone-marrow-derived mesenchymal stem cells (hBM-MSC) versus human embryonic stem cell-derived MSC (hESC-MSC) in experimental autoimmune encephalitis (EAE)—a mouse model of multiple sclerosis (MS)—Wang et al. [1] discovered that hBM-MSC failed to match hES-MSC in efficacy, identifying release of interleukin-6 (IL-6) by the hBM-MSC as a possible reason. This is important, first because—in T-lymphocyte maturation - IL-6 promotes differentiation of the inflammatory Th17 effector cell lineage [3]; and second because Th17 cells are key mediators of the autoimmune disease process [4]. Th17 cells normally function in the gut and mucosal barriers to prevent invading pathogens, but may enter the peripheral circulation and in autoimmune conditions show inappropriate activity including within the central nervous system (CNS) in patients suffering from MS. Here, Th17 cells cross the blood brain barrier and associate with inflammatory lesions of the brain and spinal cord [5]. Notably, in the EAE model, IL-6 released by hBM-MSC correlated with increased Th17 immunity and exacerbation of disease [1]. Given the links (a) IL-6 and hBM-MSC, and (b) IL-6's involvement in inflammatory Th17 immunity, the use of hBM-MSC as a source of stem cells to treat patients with MS, even for autologous grafts, may need to take into consideration potential unwanted proinflammatory effects associated with IL-6.

In contrast to IL-6, a structurally related cytokine called leukemia inhibitory factor (LIF) suppresses Th17 cells [6, 7] despite the receptors for both LIF and IL-6 sharing the gp130 receptor signaling subunit. Neural progenitor cells (NPC) derived from mouse ESC release LIF and when delivered intravenously these cells ameliorate EAE symptoms in a LIF-dependent manner that includes inhibition of Th17 in the CNS: removal of LIF by antibody blocked NPC efficacy, while replacement of NPC by recombinant LIF protein per se was efficacious [8]. Since LIF promotes Treg—a T-lymphocyte lineage absolutely required for auto-immune self-tolerance—and since activated Treg release LIF [9], Treg are likely candidates for the sustained myelin-protective auto-immunity in EAE that is linked to LIF. This accruing data suggests existence of crosstalk between therapeutic stem cells and the immune system: the stem cell-derived factors may influence regulatory mechanisms in adaptive immunity with pivotal shifts in favor of antigen-specific Treg as occurred in NPC-induced neuroprotection against EAE [8]. The dichotomy between LIF versus IL-6 in therapeutic outcome is likely to be underpinned by a critical counter-opposing regulatory LIF/IL-6 axis in T-cell differentiation, illustrated in Figure 1A. This axis in CD4+ T cells guides differentiation of either tolerogenic "Friend" Treg (LIF), or inflammatory "Foe" Th17 cells (IL-6) and is underpinned by rheostat-type control of gp190—the LIF-specific receptor subunit—at the pivotal hub. For example, IL-6 signaling shuts down expression of gp190 [6]: thus, the T cells will lose their ability to respond to LIF. Conversely, LIF signaling increases both LIF and LIF-R [6] and—critically—LIF per se directly opposes generation of new Th17 cells [8].

If long-term beneficial effects of short-lived therapeutic stem cells are indeed sustained by recipient T cells, then stability of Treg is important. Once stably committed Treg release LIF upon activation by cognate antigen, providing a Treg microenvironment biased toward self-sustaining immune tolerance that is antigen specific. Moreover, since LIF is a pleiotropic cytokine known to support stem and precursor cells including of neuronal lineage [10], the microenvironment of Treg activity will have reparative properties. Notably, LIF directly promotes reparative remyelination of demyelinated lesions within

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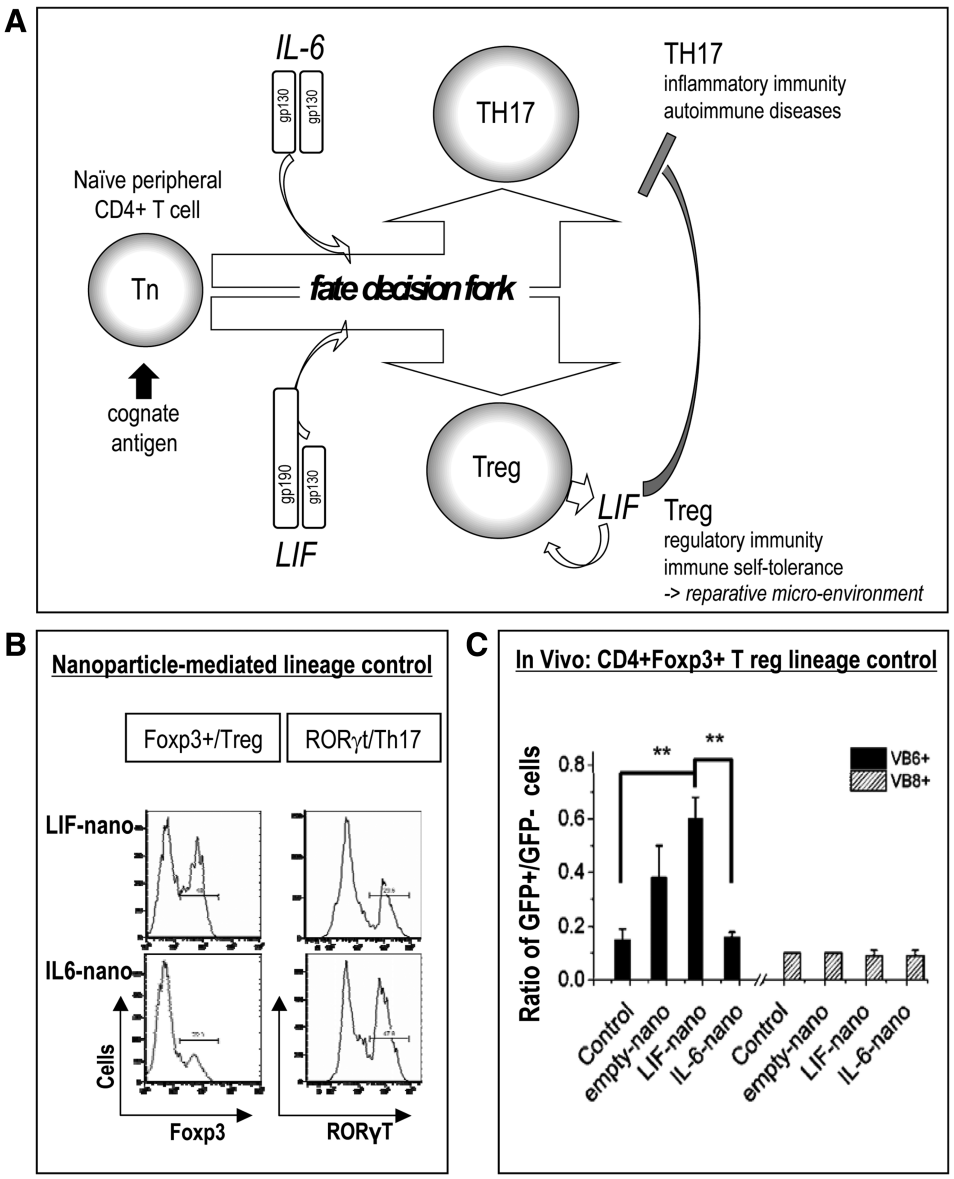

Figure 1. T-lymphocyte lineage differentiation is influenced by leukemia inhibitory factor (LIF) that has opposing effects to the influence of IL-6. **(A):** It is hypothesized that factors released by short-lived therapeutic stem cells include LIF, or closely related cytokines that signal through the LIF-receptor "gp190": increased Treg cells will sustain therapeutic benefit in an antigen-specific manner by further release of LIF [9]. This hypothesis is in accord with the recent reports (a) that short-lived stem cell grafts give rise to long-term benefits and (b) that hBM-MSC release IL-6 and are less efficacious compared to hES-MSC in reducing symptoms of EAE [1]. Notably, hBM-MSC—by releasing IL-6—may support TH17-driven immunity. In contrast, by release of LIF in response to cognate antigen, Treg both suppress TH17 cells [6, 8] and create a tolerogenic microenvironment that is also reparative for remyelination of demyelinated neurons. **(B):** LIF nanoparticles (LIF-NP) and IL-6-NP have counter-regulatory effects on Foxp3 expression in vitro as shown using Foxp3-GFP mice. Naive CD4+GFP− T cells were stimulated for 72 hours with plate-bound anti-CD3, soluble anti-CD28, soluble IL-2, and activated TGFβ. LIF-NP supported Foxp3 expression while IL-6-NP repressed Foxp3 but increased RORγt expression. **(C):** Localized delivery of LIF from CD4-targeted nanoparticles supports in vivo expansion of allo-specific Foxp3+ T cells. DBA/2 splenocytes were treated with CD4-targeted empty-NP (controls), or LIF-NP, or IL-6-NP, then infused i.v. into BALB/c Foxp3-GFP mice. At 5 days ratios of GFP+ versus GFP− host cells were calculated by fluorescence-activated cell sorting in the donor-specific Vβ6 (filled bars) or Vβ8 (striped bars) populations. Insets 1B and 1C are modified from Park et al. [7].

the CNS [11, 12]. Thus, Treg not only regulate immune tolerance but also activated Treg may impart nonimmune reparative properties within their local niche. In the Chen study [2], a rat model of immune-mediated demyelination in the CNS driven by an inflammatory neurotrophic virus revealed emergence of intraspinal Treg following intraspinal delivery of hNPC derived from hESC. These Treg were key to the therapeutic benefit initiated by the hNPC, being required to suppress neuro-inflammation, while their deletion increased neuro-inflammation, emphasizing the powerful ability of the induced Treg to overcome the combined effects of xenograft plus inflammatory neurotrophic virus within the CNS. Other examples of reparative Treg include in muscle, where a special population of muscle-specific Treg rapidly accumulate in

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acutely injured skeletal muscle, just as invading myeloid lineage cells switch from a proinflammatory to a proregenerative state [13]. In the clinical setting, patients with MS develop a novel population of circulating T cells specifically reactive to myelin basic protein and with neuro-protective properties. These cells release factors including ciliary neurotrophic factor—a cytokine that signals through the LIF-R—and arise during homeostatic recovery from lympho-depleting therapy with Alemtuzumab [14].

T lymphocyte: Friend or Foe? The evidence suggests, Friend. Powerful, immune-linked long-term benefits that carry properties of specificity and memory appear to be harnessed indirectly, downstream of short-lived therapeutic stem cells and in response to stem cell-derived factors that guide Treg maturation. Two issues arise: first, where the therapy includes immunosuppression, then conditions permissive for emergence of self-reactive Treg will be important, as designed into the "Prope tolerance" protocol first proposed by Calne et al. [15]. Second, the question arises, can growth factor alone replace use of stem cells? The potential advantages of a cell-free approach to capture the therapeutic benefits of stem cells would need to overcome issues including short half-life of factor(s) plus requirement to target therapy to sites of need. The emerging field of nanomedicine that exploits nanotechnology, including for targeted delivery of bioactive factors, is proving a promising route to harness endogenous immunity including use of lymphocyte-targeted nanoparticles carrying a cargo of LIF [7, 16] based on a nanoparticle platform already in clinical trials conducted by BIND Therapeutics.

The question arises, can LIF nanoparticles (LIF-NP) influence T cell lineage? The answer is yes. By comparing LIF-NP versus IL-6-NP attached to CD4+ T cells during their activation, Park et al. [7] showed that (a) LIF-NP induced Foxp3, required for maturation of the Treg lineage, while (b) IL-6-NP induced RORγt required for the Th17 lineage (Fig. 1B). Moreover, LIF-NP were also effective in vivo, expanding numbers of allospecific Treg in an allograft model (Fig. 1C). This is important since it confirms that the LIF/IL-6 axis can be harnessed by modulation of the cytokine milieu surrounding the targeted cell population, leading to therapeutically relevant changes in cellular homeostatic balance. The way is now open for combining nanotherapy with therapeutic stem cells where the stem cells maybe guided toward specific lineages by nanoparticle cargo as explored by Zhao et al. [17]; alternatively, therapeutic nanoparticles—for example, LIF-NP—might be targeted directly to endogenous stem cells in a cell-free approach to regenerative medicine. Moreover, and relevant to treatment of MS, low-dose LIF-NP targeted to oligodendrocyte precursor cells have been shown to promote myelin repair in vivo with high potency (Williams and Metcalfe et al., manuscript in preparation). Given (a) the data of Wang et al., and (b) that the LIF/IL-6 axis may be harnessed by nanotherapy, the notion of ex vivo treatment of hBM-MSC with LIF-NP to tip the axis away from Th17 and toward Treg following grafting would warrant consideration.

A further question concerns the role TGFβ, identified by Chen et al. as being released by hNPC and as a candidate mediator of intraspinal Treg induction [2]. In the experiments of Gao et al. [6], although activated TGFβ was required for both Treg and Th17 lineage maturation, it was LIF, versus IL-6, that imposed mutually exclusive lineage-specificity [6]. This would support a role for hNPC-derived TGFβ acting in concert with LIF, with LIF playing the pivotal role in emergence of intraspinal Treg. Overall, in the context of autoimmune inflammatory disease of the CNS, the actions of LIF—delivered either by stem cells or by nanoparticles—appear to provide threefold benefit: (a) guiding myelin-specific T-cell maturation toward self-tolerance, so reducing disease-associated autoimmune attack; (b) providing direct support for myelin repair, so reducing disease-linked neuro-degeneration; and (c) in the longer term, indirectly promoting sustained LIF within in the CNS microenvironment via CNS-resident Treg. Taken together, the findings of Wang et al. [1] and Chen et al. [2] support the concept of stem/precursor cell-derived factors leading to long-term therapeutic efficacy via T lymphocytes, and identify a need to avoid focal release of IL-6 where myelin-specific T cells are maturing.

In summary, from the immune aspect, there are two central facts to convey: (a) the proven LIF/IL-6 regulatory axis for the Treg (Friend)/Th17 (Foe) lineage maturation; and (b) that LIF directly opposes the Th17 lineage—highly relevant to treatment of MS. From the stem cell therapy aspect, long-term therapeutic outcomes are likely to be linked to crosstalk between the grafted stem cells and host immune cells, and the recent publications in Stem Cell Reports [1, 2] each independently give strong complimentary evidence of this.

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Disclosure of Potential Conflicts of Interest

The author has no conflict of interests.

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