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GIFT15 fusokine to foil immunity's follies

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Cytokines have largely been exploited in oncology to treat cancer. More specifically, granulocyte-macrophage colony-stimulating factor (GM-CSF), IFN- α and IL-2 have been routinely used in the clinic owing to their direct action on the adaptive immune system through the enhancement of antigen presentation and costimulation [1,2]. However, the weak-to-modest responses induced using these agents suggests an urgent need for the development of novel strategies to improve patients' condition. In an attempt to optimize such an approach, we developed a strategy linking innate and adaptive immunity using GM-CSF delivered as a fusion cytokine – fusokine – with other immune-stimulatory proteins. The biochemical behavior of such chimeras can lead to novel biopharmaceutical properties. For example, the fusion of GM-CSF to IL-2 (GIFT2) induces the acquisition of novel proinflammatory effects on NK cells [3]. Following the same framework, we designed a second generation GIFT fusokine combining GM-CSF to IL-15 (GIFT15) [4]. Given the fact that IL-15 shares various biological and biochemical properties with IL-2, in addition to the stimulation of both innate and adaptive immunity, we expected GIFT15 to trigger a stronger proinflammatory response than GIFT2. We were surprised to find that GIFT15 possessed immunosuppressive rather than stimulatory properties on immune cells [4]. Following further characterization, GIFT15 was found to bind aberrantly to the trimeric IL-15 receptor (IL-15R), a process probably caused by physical steric hindrance due to the GM-CSF moiety at the N-terminus of the chimera. As a result, asymmetrical signaling takes place downstream of the IL-15R, leading to the hyperactivation of STAT3, a signaling molecule known for its suppressive, proliferative, antiapoptotic and

prosurvival properties [5]. Under these conditions, it is fair to conceive that all activated lymphomyeloid cells expressing the IL-15R and exposed to GIFT15 would lose their capacity to trigger immune responses. In light of these results, we tested whether the expression of the fusokine could protect xenografted cells from rejection in immune competent animals. While mice lacking GIFT15 rejected all grafted cells, animals treated with the fusokine have tolerated the xenograft [4]. These results suggest that GIFT15 can act as a suppressor agent of allograft rejection and allows for xenotransplant as well and may present an interesting tool for the treatment of autoimmune diseases. While studying the pharmacological properties of GIFT15 on unfractionated splenocytes *in vitro*, we observed that the fusokine had a remarkable and unprecedented effect on naive B cells, converting them into suppressor cells of B-cell ontogeny (hereafter GIFT15 Bregs) [6].

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The notion of regulatory B cells (Bregs) is undergoing a revival. In the early 1970s, B cells were first suggested to play a role in immune regulation, especially after the discovery that spleen-derived B cells can suppress delayed-type hypersensitivity responses in guinea pigs [7,8]. More recently, there has been renewed interest in the notion of Bregs. Indeed, B cells have been proven to modulate autoimmunity and inflammation in numerous mouse disease models. The term Bregs was first coined by Mizoguchi and Bhan following their discovered role in the experimental autoimmune

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encephalomyelitis (EAE) and colitis [9,10]. Bregs are also known as B10 cells due to their high capacity to secrete IL-10 [11]. Upon the recognition that IL-10-producing B cells are important for the control of autoimmunity, various subsets of B cells possessing different phenotypes and origins were then identified and studied. The clearest evidence of the direct implication of Bregs in autoimmunity was first showed in the EAE mouse model of multiple sclerosis (MS). Such studies were conducted in μ MT mice, deficient in B cells, which were immunized using the MOG peptide. Interestingly, the absence of B cells did not lead to the prevention of EAE development but instead exacerbated the disease outcome [12,13]. In agreement with this notion, the adoptive transfer of wild-type B cells in μ MT mice with pre-established EAE ameliorated the disease severity whereas the transfer of *IL-10*^{-/-} B cells failed in leading to the same conclusion [12]. The main technical difficulty with the use of Bregs in the clinic lies in their relatively low abundance *in vivo* and there is no known method for the expansion of Bregs *ex vivo* due to the limited knowledge of their biology [13]. A solution to this issue will require more mechanistic insights into the development, maturation as well as activation of Bregs. Here is where GIFT15 Bregs offer an immediate and obvious remedy to this challenge and we chose to test its utility in a murine model of allergic encephalitis which recapitulates in part the pathology of MS.

“...we felt that GIFT15 Bregs would be an ideal personalized cellular medicine remedy for MS.”

Multiple sclerosis is a chronic inflammatory disorder of the CNS characterized by the activation of the immune system in a dysregulated manner against the myelin basic protein [14,15]. As a result, irreversible damages are caused to axons and their surrounding myelin sheaths. Currently, the major hypothesis explaining the appearance of MS is based on the activation or induction of some unidentified events that can lead to autoreactive T cells in periphery. Although capable of recognizing antigens from the CNS, these T cells do not leave the periphery to cross through the blood–brain barrier unless they are triggered to do so [16]. Once on site, they encounter the CNS antigen presented by resident antigen-presenting cells and become reactivated to secrete proinflammatory cytokines contributing to tissue damages. Currently, only four disease-managing therapies have been approved

for the treatment of MS: IFN- β , glatiramer acetate, mitoxantrone and the monoclonal antibody natalizumab [17]. Unfortunately, however, none of these therapies can reverse MS signs and improve patients' health. As an alternative approach, various cell therapy trials were tested in preclinical MS models, in particular the use of suppressive regulatory cells such as Tregs [18–20]. The main advantage of such an approach is the persisting suppressive effect of therapeutic cells recapitulating normal physiological suppressive pathways [18–20]. Along this line of thought, we felt that GIFT15 Bregs would be an ideal personalized cellular medicine remedy for MS.

“...a single intravenous dose of a million GIFT15 Bregs in mice symptomatic with EAE led to a complete and lasting remission in virtually all treated mice.”

In vitro tests were conducted to expand our understanding on the molecular mechanism of action of this novel GIFT15 Bregs immunosuppressive cell population. We found that GIFT15 Bregs can inhibit, as third-party bystander cells, an antigen-driven activation of T cells *in vitro* [6]. The inhibition induced by Bregs, arising from stimulation with GIFT15, differ from the Tregs based on their abundant production of the anti-inflammatory cytokine IL-10 and the upregulation of the MHC-II, a cell surface molecule known to interact with T-cell receptors. Taken together, our observations and experiments support the hypothesis that GIFT15 possesses novel biochemical properties that lead to altered affinities to the IL-15R and, thus, would be of great interest in ailments in which lymphomyeloid cells are targeted. Consistent with this idea, we sought to use GIFT15 for the generation of Bregs as a cellular biopharmaceutical to treat MS using the EAE mouse model as a proof-of-principle. Indeed, the injection of GIFT15 Bregs in EAE mice efficiently blocked stimulated T cells and stopped inflammation in spinal cords leading to their recovery within 4 weeks of treatment [6]. No apparent infiltration of lymphomyeloid cells could be detected in their spinal cords suggesting an efficient blockade of pathological reactions in the CNS. Control animals on the other hand, showed loss of muscle control arising from inflammatory nerve damage as highlighted by high average disease score. Thus, a single intravenous dose of a million GIFT15 Bregs in mice symptomatic with EAE led to a complete and lasting remission in virtually all treated mice.

All these pharmacological properties represent an attractive strategy for the use of the GIFT15 protein as a means to generate *ex vivo* cellular pharmaceuticals for the treatment of MS or other autoimmune ailments. In addition, being the host's own cells, the generated GIFT15 Bregs would not trigger an immune response that would lead to their immune rejection.

In summary, a synthetic chimeric fusokine such as GIFT15 displays remarkable and unheralded immunomodulatory properties which could not have been predicted based on the known biochemistry of its parental GM-CSF and IL-15 domains. Indeed, such as an approach

– linking biologically active proteins in an unnatural manner – may be a strategy that confuses Mother Nature and allows us to foil her pathological tantrums.

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Bibliography

- 1 Metcalf D: Hematopoietic cytokines. *Blood* 111, 485–491 (2008).
- 2 McDermott DF: Immunotherapy of metastatic renal cell carcinoma. *Cancer* 115(10 Suppl.), 2298–2305 (2009).
- 3 Stagg J, Wu JH, Bougamin N *et al.*: Granulocyte-macrophage colony-stimulating factor and interleukin-2 fusion of cDNA for cancer gene immunotherapy. *Cancer Res.* 64, 8795–8799 (2004).
- 4 Rafei M, Wu JH, Annabi B *et al.*: A GM-CSF and IL-15 fusokine leads to paradoxical immunosuppression *in vivo* via asymmetrical JAK/STAT signaling through the IL-15 receptor complex. *Blood* 109, 2234–2242 (2007).
- 5 Brantley EC, Benveniste EN: Signal transducer and activator of transcription-3: a molecular hub for signaling pathways in gliomas. *Mol. Cancer Res.* 6, 675–684 (2008).
- 6 Rafei M, Hsieh J, Zehntner S *et al.*: A GM-CSF/interleukin-15 fusokine leads to the generation of a type of immune regulatory B-cell with potent immune suppressive properties as demonstrated in the EAE mouse model of multiple sclerosis. *Nat. Med.* (2009) (In press).
- 7 Katz SI, Parker D, Turk JL: B-cell suppression of delayed hypersensitivity reactions. *Nature* 251, 550–551 (1974).
- 8 Neta R, Salvin SB: Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. *J. Immunol.* 113, 1716–1725 (1974).
- 9 Mizoguchi A, Bhan AK: A case for regulatory B cells. *J. Immunol.* 176, 705–710 (2006).
- 10 Mauri C, Ehrenstein MR: The 'short' history of regulatory B cells. *Trends Immunol.* 29, 34–40 (2008).
- 11 Haas KM, Poe JC, Steeber DA, Tedder TF: B-1a and B-1b cells exhibit distinct developmental requirements and have unique functional roles in innate and adaptive immunity to *S. pneumoniae*. *Immunity* 23, 7–18 (2005).
- 12 Fillatreau S, Sweeney CH, McGeachy MJ, Gray D, Anderton SM: B cells regulate autoimmunity by provision of IL-10. *Nat. Immunol.* 3, 944–950 (2002).
- 13 Bouaziz J-D, Yanaba K, Tedder TF: Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol. Rev.* 224, 201–214 (2008).
- 14 Williams KC, Ulvestad E, Hickey WF: Immunology of multiple sclerosis. *Clin. Neurosci.* 2, 229–245 (1994).
- 15 Kuhlmann T, Remington L, Cognet I *et al.*: Continued administration of ciliary neurotrophic factor protects mice from inflammatory pathology in experimental autoimmune encephalomyelitis. *Am. J. Pathol.* 169, 584–598 (2006).
- 16 Jack C, Ruffini F, Bar-Or A, Antel JP: Microglia and multiple sclerosis. *J. Neurosci. Res.* 81, 363–373 (2005).
- 17 Hartung HP, Bar-Or A, Zoukos Y: What do we know about the mechanism of action of disease-modifying treatments in MS? *J. Neurol.* 251(Suppl. 5), V12–V29 (2004).
- 18 Stern JN, Keskin DB, Zhang H, Lv H, Kato Z, Strominger JL: Amino acid copolymerspecific IL-10-secreting regulatory T-cells that ameliorate autoimmune diseases in mice. *Proc. Natl Acad. Sci.* 105, 5172–5176 (2008).
- 19 Giorgini A, Noble A: Blockade of chronic graft-versus-host disease by alloantigeninduced CD4⁺CD25⁺Foxp3⁺ regulatory T-cells in nonlymphopenic hosts. *J. Leukoc. Biol.* 82, 1053–1061 (2007).
- 20 Karim M, Feng G, Wood KJ, Bushell AR: CD25⁺CD4⁺ regulatory T-cells generated by exposure to a model protein antigen prevent allograft rejection: antigen-specific reactivation *in vivo* is critical for bystander regulation. *Blood* 105, 4871–4877 (2005).