

T Cell-Derived GM-CSF, Regulation of Expression and Function

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Abstract

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is produced by a variety of cells and plays an important role in the inflammatory response in infection as well as in autoimmunity. Recent progress has indicated that CD4⁺ T cell-derived GM-CSF has a prominent and non-redundant function in mediating autoimmune neuroinflammation. Thus, there is increased interest in the regulation of GM-CSF production by T helper cells, which could translate to the development of novel therapeutics for autoimmune diseases such as multiple sclerosis. This review focuses on our current understanding of the regulation and function of T cell-derived GM-CSF.

Keywords: GM-CSF; Experimental autoimmune encephalomyelitis; Multiple sclerosis; T helper cells; TH17; TH-GM

Introduction

Granulocyte-macrophage colony-stimulating factor (GM-CSF), also known as colony stimulating factor 2 (CSF2), was termed due to its ability to promote *in vitro* differentiation of granulocytic and macrophage colonies from bone marrow precursors and is therefore considered as a hematopoietic-cell growth factor. However, GM-CSF-null mice undergo normal hematopoiesis and only the maturation of alveolar macrophages is compromised [1]. GM-CSF is produced by various types of cells, including activated T/B cells, monocytes/macrophages, neutrophils, fibroblasts, epithelial cells, endothelial cells, stromal cells and tumor cells [2]. An increasing body of evidences supports GM-CSF as a potent proinflammatory factor that is critically involved in inflammatory and autoimmune conditions [2,3] and that the function of this cytokine varies depending on the origin of the cytokine and the status of the cells *in vivo*. CD4⁺ T cells are critical for immune response against microbial infection but also play essential roles in autoimmunity. Naïve CD4⁺ T cells can differentiate into various subsets of T helper (TH) cells including TH1, TH2 and TH17 cells. Each subset of TH cells expresses unique cytokines and has distinct functions in immunity. Interestingly, GM-CSF is more or less expressed by all of these TH cells. In this review, we will focus on the recent discoveries concerning the function of CD4⁺ T cell-derived GM-CSF as well as the regulatory mechanism governing GM-CSF expression by CD4⁺ T cells.

TH cell-derived GM-CSF in autoimmune neuronal disease

For decades it has been known that activated T cells are capable of producing GM-CSF [4]. However, it was not until recently that the functional importance of T cell-derived GM-CSF was identified. Antigen-specific CD4⁺ TH cells are primary effectors in many inflammatory and autoimmune diseases; including the progressive

demyelinating disease multiple sclerosis (MS) and its rodent model experimental autoimmune encephalomyelitis (EAE) [5]. Studies based on the pathogenesis of MS and EAE have greatly contributed to the discovery and functional understanding of the various CD4⁺ T cell subsets. Originally, it was believed that TH1 cells producing IFN γ were the major contributor to the pathogenesis of MS. However, this hypothesis was conclusively refuted following the identification of IL-23 as a major player in EAE [6,7]. Mice lacking IL-23 (p19/p40), but not the TH1 promoting cytokine IL-12 (p35/p40), were found to be resistant to myelin oligodendrocyte glycoprotein (MOG)-induced EAE [6]. IL-23 was found to promote the development and expansion of IL-17-producing CD4⁺ T cells co-expressing IL-17F, IL-6 and TNF. In addition, IL-23-driven, IL-17-producing autoreactive T cells are highly pathogenic and essential for the establishment of autoreactive central nervous system autoimmunity [7]. Subsequently, a novel TH cell subset expressing IL-17 and IL-17F but not IFN γ or IL-4, was identified (Park, 2005; Harrington, 2005). The discovery of the TH17 lineage dramatically changed the TH1-TH2 CD4⁺ T helper cell paradigm and triggered enormous interest in the biology of this TH subset of cells in the field of immunology. After years of careful characterization, it was found that IL-23 is not necessary for the initiation of TH17 cell differentiation [8]. Instead, IL-23 promotes stability and terminal differentiation of TH17 cells [9].

The TH17 cells are believed to play important roles in various inflammatory and autoimmune diseases including MS and rheumatoid arthritis (RA) [10,11]. However, the main cytokines produced by TH17 cells were found to be nonessential in EAE. Mice deficient in IL-17A are fully susceptible to EAE and overexpression of IL-17A in T cells has no impact on the pathogenesis of EAE [12]. Similarly, mice deficient in IL-17F are fully susceptible to EAE and removing both IL-17A and IL-17F in the course of EAE does not significantly impact the development of EAE in mice. Both IL-21 and IL-22, two cytokines also produced by TH17 cells, are also not required for EAE development [13,14]. Subsequently, it was found

instead that the GM-CSF produced by TH17 cells had an essential role in their encephalitogenicity [15].

The importance of GM-CSF in inflammation and inflammatory diseases including EAE and arthritis had been demonstrated by studies using mice deficient in GM-CSF well before the discovery of TH17 cells [16,17]. GM-CSF-deficient mice are resistant to both collagen-induced arthritis and MOG-induced EAE [17]. Upon induction of EAE, GM-CSF knockout (KO) mice developed much less inflammatory lesions in the CNS compared to wild-type (WT) mice [17]. In addition, unlike the WT mice, the KO mice failed to sustain such inflammatory lesions in CNS. As such, little disease was observed in the KO mice. These findings suggested that although the autoreactive cells in the GM-CSF KO mice were able to migrate into the CNS they fail to mount sustained inflammation, which could be indicative of a reduction in cell number, impaired encephalitogenicity or both. To further understand how GM-CSF regulates the disease process of EAE, Marusic et al. overexpressed GM-CSF in MBP-specific T cells using a retroviral-mediated approach and then adoptively transferred GM-CSF-overexpressing cells into mice for EAE induction [18]. Mice receiving both control MBP-specific cells or GM-CSF-overexpressing cells developed EAE disease within similar time period. However, mice receiving GM-CSF-overexpressing cells developed a more severe and chronic form of EAE that could not be resolved, indicating that autoreactive T cells expressing GM-CSF in the CNS play important roles in the progression of disease severity. Years later, it was confirmed that GM-CSF produced by antigen-specific T cells, but not CNS resident or other peripheral cells, was required for EAE induction [19]. In this case, WT MBP-specific T cells potently transferred EAE disease in GM-CSF-null mice, whereas GM-CSF-deficient T cells failed to do so. In addition, it was found that only the activation of microglial cells is GM-CSF-dependent and the activation of peripheral macrophages was not affected. Together, these studies demonstrated that GM-CSF derived from encephalitogenic T cells is critical for the activation of microglial cells, the initiation of EAE, and to sustain inflammatory response in the CNS, thereby significantly influencing the rate of disease development and severity.

The important role of T cell-derived GM-CSF in EAE was further supported by studies from two groups that independently reported that GM-CSF secreted by IFN- γ -deficient or IL-17-deficient CD4⁺ T cells was sufficient for the induction of EAE [15,20]. In addition, disruption of NF- κ B2, a component of the noncanonical NF- κ B pathway, resulted in impaired GM-CSF expression by inflammatory T cells and protected mice from EAE [21]. Deficiency of Bhlhe40, a helix-loop-helix transcription factor that is required for GM-CSF expression in effector T cells, also resulted in resistance to MOG-induced EAE [22,23]. In addition, deletion of either STAT5 or STAT4 leads to impaired GM-CSF production by CD4⁺ T cells and caused resistance to EAE [24,25]. Together, these studies further demonstrate the importance of T cell-derived GM-CSF in the pathogenesis of neuronal autoreactive disease.

The critical role of T cell-derived GM-CSF in autoimmune inflammation has been further substantiated by studies in human MS. Noster et al. reported that increased GM-CSF-producing CD4⁺ T cells correlate to MS incidence. These cells were particularly found to be elevated in the cerebrospinal fluid of active MS patients compared with patients with noninflammatory neurological pathologies (OND) [26]. The association between GM-CSF-producing CD4⁺ T cells and active MS was further supported by another report showing that the frequency of GM-CSF-producing CD4⁺ T cells, but not CD8⁺ T cells,

was significantly increased in MS patients compared to patients with OND. Outside of incidence, the frequency of GM-CSF-producing CD4⁺ in MS is also associated with disease severity [27]. IFN- β , a drug prescribed to control MS progression, may function in part through suppressing GM-CSF expression by peripheral blood T cells of MS patients [28]. Of note, however, there is a discrepancy in these reports as to whether the frequency of GM-CSF-producing CD8⁺ T cells was also elevated in MS patients [27,28]. Nevertheless, it is likely that CD4⁺ T cell-derived GM-CSF plays a pivotal role in the pathogenesis of MS and might be a promising target for therapeutic intervention. The level of GM-CSF expression by CD4⁺ T cells in peripheral blood also has potential to be used as a biomarker for diagnosis and prognosis of MS.

In addition to EAE and MS, several studies had shown a non-redundant function of T cell-derived GM-CSF in various types of inflammatory and autoimmune disorders. For instance, GM-CSF production by CD4⁺ T cells was reported to promote differentiation and proliferation of Th17 cells indirectly through inducing IL-6 secretion by dendritic cells, a process contributing to mouse myocarditis [29]. CD4⁺ T cell-secreted GM-CSF was required for the generation of monocyte-derived inflammatory dendritic cells (infDCs) and disease progression for acute inflammatory arthritis and peritonitis in mice [30]. In human rheumatoid arthritis (RA), synovial CD4⁺ T cells were found to be a major source of GM-CSF that strongly promoted infDC differentiation [31]. In juvenile idiopathic arthritis, the frequency of GM-CSF-producing CD4⁺ T cells in synovial fluid was significantly correlated with GM-CSF protein level as well as serum erythrocyte sedimentation rate, an indicator of inflammation [32]. In a mouse model of human interstitial lung disease (ILD), there was increased infiltration of both GM-CSF-producing CD4⁺ T cells and TH17 cells into lung tissues [33]. Neutralization of GM-CSF, but not IL-17A, blocked the development of ILD, supporting a requirement of GM-CSF in disease development.

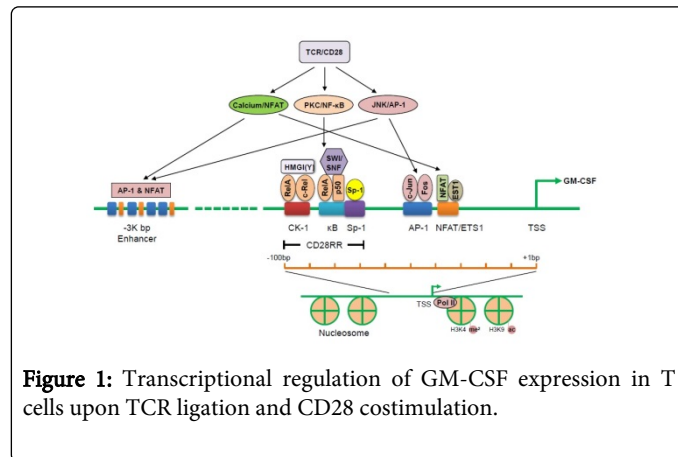
GM-CSF has been shown to be broadly involved in many other autoimmune/inflammatory diseases, including nephritis [34,35], atherosclerosis [36,37], diabetes [38,39], obesity [40], and Crohn's disease [41-43]. However, in most of these studies, the exact cellular source of GM-CSF has yet to be identified.

Together, these studies demonstrated the importance of GM-CSF in the pathogenesis of various inflammatory and autoimmune diseases. Hence, recent studies have focused on the development of treatment regime based on targeting of GM-CSF in inflammatory conditions such as RA [2,44]. Since CD4⁺ T cells were found to be a major source of GM-CSF in many of these inflammatory diseases, it will be of great interest to understand and characterize GM-CSF-producing T cells for future clinical applications

Transcriptional regulation of GM-CSF expression in CD4⁺ T cells upon TCR activation and CD28 costimulation

Without activation, the expression of GM-CSF by T cells, similar to the expression of other cytokines such as IL-2 and IFN, is minimal. T cell receptor ligation and CD28 costimulation rapidly, but also transiently, induces GM-CSF transcription [45]. The expression of GM-CSF itself is regulated by a proximal promoter and an upstream enhancer [46]. The proximal promoter of Csf2 gene has a CD28 response region (CD28RR, -102~-69 bp relative to the transcription start site) containing a 10 bp element known as CD28 response element (CK-1 in the GM-CSF promoter or 28RE in the IL-2 promoter, -101~-92 bp), which is a variant NF κ B binding site (Figure

1). The RelA and c-Rel transcriptional factors, which are NF- κ B elements, bind CK-1 with assistance from the high mobility group of protein HMGI(Y) to regulate the activity of the CD28 response element [47]. NFAT proteins, in response to the TCR/CD28-mediated calcium cascade, bind to the CK-1 element to potentiate the activity of the GM-CSF CD28RR [48]. However, the binding of NFATp and Rel proteins appears to be mutually exclusive.



TCR ligation and CD28 costimulation activate GM-CSF expression in T cells via three major pathways including PKC/NF- κ B, JNK/AP-1 and Calcium/NFAT pathways. NF- κ B proteins bind to the CD28 response region (CD28RR), which consists of CK-1 and κ B/Sp-1 elements within the proximal promoter, and recruit SWI/SNF chromatin remodeling complexes to reposition nucleosome to increase chromatin accessibility and to induce transcription of Csf2 gene. AP-1 and NFAT proteins bind to a region downstream of the κ B/SP-1 element within proximal promoter and the inducible enhancer to transactivate GM-CSF expression. Epigenetic modifications (such as H3K4me² and H3K9ac) are also implicated in regulating the transcriptional activity of Csf2 locus.

The CK-1/CD28RE elements do not function as isolated elements but require adjacent transcription factor binding sites in the CD28RR for activity [48]. An adjacent region downstream of CK-1 contains a classical NF- κ B site (binds to p50/RelA complex), a Sp-1 site, and a putative AP-1 site, which are also required for maximal responses to CD28 signaling and optimal transcription of GM-CSF. The function of the adjacent NF- κ B and Sp-1 sites was illustrated in mice that carry a human GM-CSF transgene containing mutations in the NF- κ B/Sp-1 sites within the CD28RR [49]. Mutations in the NF- κ B site reduced its binding to p50/RelA complex and to Sp-1, disrupted chromatin remodeling of the promoter, and resulted in reduced expression of GM-CSF. Changes of chromatin structure or chromatin remodeling are important for the permissive transcription of inducible genes such as GM-CSF [50]. NF- κ B proteins appear to be important for chromatin remodeling at the GM-CSF promoter in T cells upon TCR activation. NF- κ B proteins recruit SWI/SNF chromatin remodeling complexes to CD28RR, which requires both the CK-1 and the classical NF- κ B sites and leads to high chromatin accessibility across Csf2 promoter upon T cell activation [50]. More recently, it was shown that NF- κ B1 [p50] may form homodimers or heterodimers with c-Rel, which potentially could induce GM-CSF expression in activated CD4⁺ T cells [30]. Another region (-54~-31 bp) within the proximal promoter of Csf2 that contains NFAT, AP-1, and ETS1 binding sites also regulates GM-CSF promoter activity [5,51,52]. Although the

calcium/NFAT pathway is not necessary for promoter remodeling, both PKC/NF- κ B1 and calcium/NFAT pathways are required for efficient Csf2 transcription [53].

An inducible enhancer located at 3k bp upstream of transcription start site of Csf2 contains binding sites for NFAT and AP-1 proteins, and integrates multiple signals for the control of GM-CSF expression as well [54,55]. This enhancer may contain an inducible DNase I hypersensitive site and the deletion of this enhancer led to a significantly decreased expression of human GM-CSF transgene in mice [56]. The cooperation between NFAT and AP-1 directs chromatin remodeling and increases accessibility to this inducible enhancer in T cells [57]. Further studies revealed that this inducible enhancer is differentially regulated by GATA and NFAT elements/proteins in different types of cells via directing distinct patterns of nucleosome positioning [58]. Inhibition of GM-CSF enhancer activity by glucocorticoid, which activates glucocorticoid receptor and competes with the NFAT/AP-1 for binding to the GM-CSF enhancer, results in suppression of GM-CSF expression by T cells [59]. Epigenetic regulation tightly controls the Csf2/IL3 loci throughout the course of T cell differentiation and activation [60]. Csf2 locus is epigenetically silent in CD4/CD8 double-positive thymocytes, but can acquire active epigenetic modifications linked to RNA Pol II recruitment and transcriptional activation following T cell activation and maturation [60].

Regulation of TH17-derived GM-CSF

GM-CSF is known to be produced by activated T cells and it is clear that T cell-derived GM-CSF is essential for the autoimmune inflammation. However, it still remains controversial whether there is a specific TH cell subset that produces GM-CSF and plays a more dominant role in such diseases. Moreover, the factors that induce GM-CSF expression during or after T cell differentiation are still unclear. IL-23 was found to stimulate GM-CSF expression in TH17 cells [15,20]. El-Behi et al. demonstrated that both antigen-specific TH1 and TH17 are encephalitogenic and that GM-CSF expression by either TH1 or TH17 cells was required for their encephalitogenicity in mice [15]. IL-23 promotes GM-CSF expression in TH17 cells, whereas TGF β inhibits its expression. Moreover, TH17 cell-derived GM-CSF stimulates IL-23 expression by antigen presenting cells (APCs), which in turn to stimulate TH17 cells to express GM-CSF, thereby forming a positive feedback loop to sustain and augment neuronal inflammation. Furthermore, it was found that both T-bet and ROR γ t are not required for GM-CSF expression. Codarri et al. showed that GM-CSF marked a population of highly pathogenic helper T cells that plays a non-redundant role in neuro-autoimmune pathogenicity in mice [20]. By adoptive transfer of MOG-specific cells polarized to secrete IFN γ , IL-17 or GM-CSF, Codarri et al. found that although all the three type of cells were able to initiate EAE development, mice receiving cells polarized to secrete GM-CSF had significantly earlier onset of the disease and greater disease severity than the recipients of cells polarized to secrete IFN γ or IL-17. The generation of this highly pathogenic GM-CSF-producing TH population was driven by IL-23 but inhibited by IFN γ , IL-12, and IL-27. Interestingly, ROR γ t was found to be required for TH cells for the expression of GM-CSF. El-Behi et al. found that other than IL-23, IL-1 β was also stimulatory for GM-CSF expression in TH1 and TH17 cells [15], which was consistent with a report from Lukens et al. showing that inflammasome-derived IL-1 β signals through IL-1R-MyD88 axis to promote GM-CSF expression by CD4⁺ T cells [61]. However, the mechanism by which

IL-23 or IL-1 β stimulates GM-CSF expression in TH cells remains unclear.

Studies mentioned above, together with others, demonstrated that both TH1 and TH17 cells were able to mediate EAE development in mice and GM-CSF expression is required for their encephalitogenicity. IL-23 promotes GM-CSF expression via an unknown mechanism. GM-CSF therefore links both TH1 and TH17 cells to EAE and provides an answer for the question why in EAE, the signature cytokines of TH1 or TH17 cells were not required, whereas IL-23 is important.

The Emergence of GM-CSF-only-producing TH cells in humans and rodents

In addition to GM-CSF-expression by TH1, TH2 and TH17 cells, a GM-CSF-only-producing CD4⁺ T cell population associated with autoimmune neuronal inflammation was identified in both humans and mice [25,26]. It was found that in human TH cells, TH1 cells but not TH17 cells were the main producers of GM-CSF [26]. Cells co-expressing GM-CSF in addition to IL-17, IL-4 or IL-22 were also found. In addition, a GM-CSF-only-producing cell population was identified, which constituted only 2.2% of the entire CD4⁺ TH cell population in healthy donors. The GM-CSF-only-producing cells, which lack IFN γ , IL-17, IL-4 or IL-22, were subsequently cloned and examined. The authors found that the expression of T-bet, GATA3 or ROR γ t was minimal in these cells, indicating that their transcriptional regulation is different from that of TH1, TH2 or TH17 cells [26]. Differentiation conditions for human (combination of IL-1 β and IL-6) or mouse TH17 cells (combination of IL-1 β , IL-6 and IL-23, or IL-6 and TGF β) suppressed the polarization of human GM-CSF-only-producing cells. IL-23, which was previously shown to promote GM-CSF production by TH1 or TH17 cells in mice, was inhibitory for GM-CSF production in humans. The inhibitory effect of IL-6, IL-23 and IL21 on GM-CSF expression was found to be STAT3-dependent. Similarly, ROR γ t was found to be inhibitory for the generation of GM-CSF-producing cells in humans. On the other hand, IL-12 promotes GM-CSF production in a STAT4-dependent manner. Furthermore, IL-2 signaling through STAT5 was able to promote TH GM-CSF production. These findings demonstrated that GM-CSF expression by human TH cells differentially regulated at the transcriptional level compared to TH17 cells (Figure 2).

Upon T cell activation, cytokine signals are crucial in directing the differentiation of different subsets of T_H cells. IL-2 in human (Figure 2A) or IL-7 in mouse (Figure 2B) binds to γ_c -containing receptors and triggers the differentiation of GM-CSF-only-producing termed T_H-GM cells. STAT5 proteins are key transmitters of IL-2 or IL-7 receptor signals therefore critically regulate T_H-GM differentiation from naïve CD4⁺ T cells of both human and mouse origins, likely through directly binding to cis-elements within csf2 promoter. Autocrine IL-2 secreted by differentiating TH-GM cells may reinforce GM-CSF expression. In addition, mouse IL-3 expression appears to be co-regulated with GM-CSF by IL-7/STAT5 signaling. Several other cytokines including IL-1 β , IL6, IL-12 and IL-23 can either activate or suppress GM-CSF expression in mouse or human T_H-GM as depicted.

Similarly, GM-CSF-only-producing TH cells were identified in mice [25]. The differentiation conditions of the mouse GM-CSF-only-producing TH cells, termed TH-GM, from naïve CD4⁺ T cells was different from that of TH1 or TH17 cells. It was found that TH1 differentiation conditions (IL-12 and anti-IL-4) or TH17 differentiation conditions (TGF β and IL-6 or IL-6, IL-23 and IL-1 β in combination with blocking both IFN γ and IL-4) inhibits GM-CSF expression. Moreover, inhibiting IFN γ , IL-4, and IL-6 promoted the generation of TH-GM cells while IL-23 and IL1 β had no effect. Importantly, IL-7 was found to promote the generation of TH-GM cells in a STAT5-dependent manner. This is confirmed as IL-7 augmented the generation of TH-GM cells in a dose-dependent manner from WT naïve CD4⁺ T cells but not from STAT5-deficient naïve CD4⁺ T cells. Mice with T-cell-specific deletion of STAT5 were resistant to MOG-induced EAE, which was associated with reduced GM-CSF-expressing T cells, but not IFN γ - or IL-17-producing T cells in CNS. In addition, STAT5-deficient CD4⁺ T cells from MOG-immunized mice were impaired in inducing EAE after passive transfer to Rag2^{-/-} mice. IL-2, which also signals through STAT5, did not promote the generation of TH-GM cells in mice. Furthermore, mouse TH-GM cells were found to have a transcriptional program distinct from that of TH1 or TH17 cells and could more effectively transfer EAE to Rag2^{-/-} mice compared to TH1 or TH17 cells [25]. Together, these findings demonstrated the existence of TH-GM cells with important role in the pathogenesis of autoimmune neuronal disease.

Conclusion

Evidence from both human and animal studies have conclusively demonstrated that T cell-derived GM-CSF, regardless of T cell subset sources, plays a non-redundant role in the pathogenesis of multiple autoimmune inflammatory diseases such as EAE and MS. Moreover, a new subset of CD4⁺ T cells, TH-GM cells, is emerging as a new player contributing to neuronal inflammation. Interestingly, the differentiation conditions controlling TH17 cell generation also suppresses TH-GM cell generation in both humans and mice in vitro. Thus, the relatively new paradigm of GM-CSF-producing TH17 cells as the primary trigger for MS/EAE may be subsequently challenged in the near future.

STAT5 activation promotes the differentiation of TH-GM cells, whereas STAT3 activation is inhibitory. Consequently, studies using STAT5 T cell-specific KO mice have demonstrated that mouse TH-GM cells are important for the initiation and maintenance of inflammation in CNS [25]. Given that multiple subsets of TH cells including TH1, TH17 and TH-GM are able to produce GM-CSF, the relative importance of TH-GM cells in MS is still unclear. Furthermore, TH-GM cells in humans constitute only about 2% of the

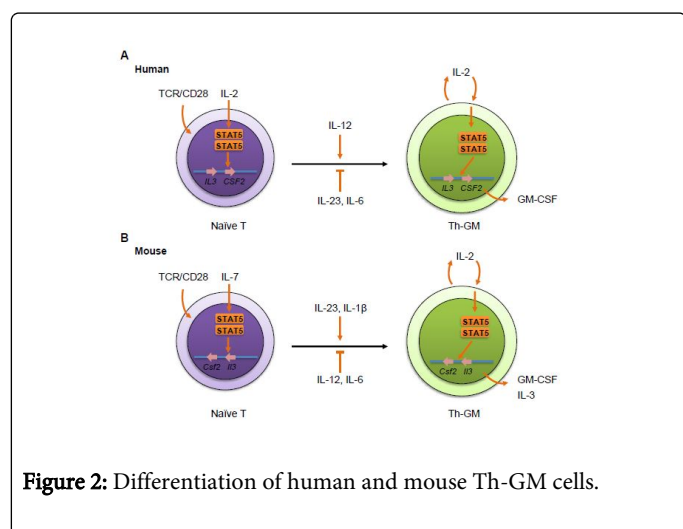


Figure 2: Differentiation of human and mouse Th-GM cells.

total CD4⁺ T cell population in blood from healthy individuals [26], whereas the relative frequency of mouse TH-GM is not clear. The idea of TH-GM cells is still in its infancy and it remains to be determined whether these cells are capable of producing additional mediators that could augment the effects of GM-CSF and further contribute to inflammatory pathogenicity. Furthermore, an expanded characterization of GM-CSF-producing CD4⁺ T cells, including biomarker expression on the cellular surface, will lead to more robust studies to determine the viability and efficacy of targeting this cell type for the treatment of inflammatory disease. Thus, several knowledge gaps still exist and further research should be focused on persistence of these cells in other disease conditions, how these cells are developed in vivo, and how their inflammatory function is regulated at the transcriptional and epigenetic level. This in turn, will be highly beneficial for the complete characterization of these cells in human health and disease, which can be translated for clinical applications.

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References

1. Stanley E, Lieschke GJ, Grail D, Metcalf D, Hodgson G, et al. (1994) Granulocyte/macrophage colony-stimulating factor-deficient mice show no major perturbation of hematopoiesis but develop a characteristic pulmonary pathology. *Proc Natl Acad Sci USA* 91: 5592-5596.
2. Hamilton JA (2008) Colony-stimulating factors in inflammation and autoimmunity. *Nat Rev Immunol* 8: 533-544.
3. Hamilton JA (2015) GM-CSF as a target in inflammatory/autoimmune disease: current evidence and future therapeutic potential. *Expert Rev Clin Immunol* 11: 457-465.
4. Griffin JD, Cannistra SA, Sullivan R, Demetri GD, Ernst TJ, et al. (1990) The biology of GM-CSF: regulation of production and interaction with its receptor. *Int J Cell Cloning* 8 Suppl 1: 35-44.
5. Emerson MR, Gallagher RJ, Marquis JG, LeVine SM (2009) Enhancing the ability of experimental autoimmune encephalomyelitis to serve as a more rigorous model of multiple sclerosis through refinement of the experimental design. *Comparative medicine* 59: 112-128.
6. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, et al. (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421: 744-748.
7. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, et al. (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201: 233-240.
8. Dong C (2008) TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol* 8: 337-348.
9. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, et al. (2009) The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 10: 314-324.
10. Waisman A, Hauptmann J, Regen T (2015) The role of IL-17 in CNS diseases. *Acta Neuropathol* 129: 625-637.
11. Lubberts E (2015) The IL-23-IL-17 axis in inflammatory arthritis. *Nat Rev Rheumatol* 11: 415-429.
12. Haak S, Croxford AL, Kreyenborg K, Heppner FL, Pouly S, et al. (2009) IL-17A and IL-17F do not contribute vitally to autoimmune neuroinflammation in mice. *J Clin Invest* 119: 61-69.
13. Kreyenborg K, Ezensperger R, Dumoutier L, Haak S, Rebollo A, et al. (2007) IL-22 is expressed by Th17 cells in an IL-23-dependent fashion, but not required for the development of autoimmune encephalomyelitis. *J Immunol* 179: 8098-8104.
14. Coquet JM, Chakravarti S, Smyth MJ, Godfrey DI (2008) Cutting edge: IL-21 is not essential for Th17 differentiation or experimental autoimmune encephalomyelitis. *J Immunol* 180: 7097-7101.
15. El-Behi M, Ciric B, Dai H, Yan Y, Cullimore M, et al. (2011) The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. *Nat Immunol* 12: 568-575.
16. Campbell IK, Rich MJ, Bischof RJ, Dunn AR, Grail D, et al. (1998) Protection from collagen-induced arthritis in granulocyte-macrophage colony-stimulating factor-deficient mice. *J Immunol* 161: 3639-3644.
17. McQualter JL, Darwiche R, Ewing C, Onuki M, Kay TW, et al. (2001) Granulocyte macrophage colony-stimulating factor: a new putative therapeutic target in multiple sclerosis. *J Exp Med* 194: 873-882.
18. Marusic S, Miyashiro JS, Douhan J 3rd, Konz RF, Xuan D, et al. (2002) Local delivery of granulocyte macrophage colony-stimulating factor by retrovirally transduced antigen-specific T cells leads to severe, chronic experimental autoimmune encephalomyelitis in mice. *Neurosci Lett* 332: 185-189.
19. Ponomarev ED, Shriver LP, Maresz K, Pedras-Vasconcelos J, Verthelyi D, et al. (2007) GM-CSF production by autoreactive T cells is required for the activation of microglial cells and the onset of experimental autoimmune encephalomyelitis. *J Immunol* 178: 39-48.
20. Codarri L, Gyölvézi G, Tosevski V, Hesse L, Fontana A, et al. (2011) ROR γ t drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. *Nat Immunol* 12: 560-567.
21. Yu J, Zhou X, Nakaya M, Jin W, Cheng X, et al. (2014) T cell-intrinsic function of the noncanonical NF- κ B pathway in the regulation of GM-CSF expression and experimental autoimmune encephalomyelitis pathogenesis. *J Immunol* 193: 422-430.
22. Martínez-Llordella M, Esensten JH, Bailey-Bucktrout SL, Lipsky RH, Marini A, et al. (2013) CD28-inducible transcription factor DEC1 is required for efficient autoreactive CD4⁺ T cell response. *J Exp Med* 210: 1603-1619.
23. Lin CC, Bradstreet TR, Schwarzkopf EA, Sim J, Carrero JA, et al. (2014) Bhlhe40 controls cytokine production by T cells and is essential for pathogenicity in autoimmune neuroinflammation. *Nat Commun* 5: 3551.
24. McWilliams IL, Rajbhandari R, Nozell S, Benveniste E, Harrington LE (2015) STAT4 controls GM-CSF production by both Th1 and Th17 cells during EAE. *J Neuroinflammation* 12: 128.
25. Sheng W, Yang F, Zhou Y, Yang H, Low PY, et al. (2014) STAT5 programs a distinct subset of GM-CSF-producing T helper cells that is essential for autoimmune neuroinflammation. *Cell Res* 24: 1387-1402.
26. Noster R, Riedel R, Mashreghi MF, Radbruch H, Harms L, et al. (2014) IL-17 and GM-CSF expression are antagonistically regulated by human T helper cells. *Sci Transl Med* 6: 241ra80.
27. Hartmann FJ, Khademi M, Aram J, Ammann S, Kockum I, et al. (2014) Multiple sclerosis-associated IL2RA polymorphism controls GM-CSF production in human TH cells. *Nat Commun* 5: 5056.
28. Rasouli J, Ciric B, Imitola J, Gonnella P, Hwang D, et al. (2015) Expression of GM-CSF in T Cells Is Increased in Multiple Sclerosis and Suppressed by IFN β Therapy. *J Immunol* 194: 5085-5093.
29. Sonderegger I, Iezzi G, Maier R, Schmitz N, Kurrer M, et al. (2008) GM-CSF mediates autoimmunity by enhancing IL-6-dependent Th17 cell development and survival. *J Exp Med* 205: 2281-2294.
30. Campbell IK, van Nieuwenhuijze A, Segura E, O'Donnell K, Coghill E, et al. (2011) Differentiation of inflammatory dendritic cells is mediated by NF- κ B1-dependent GM-CSF production in CD4⁺ T cells. *J Immunol* 186: 5468-5477.
31. Reynolds G, Gibbon JR, Pratt AG, Wood MJ, Coady D, et al. (2015) Synovial CD4⁺ T-cell-derived GM-CSF supports the differentiation of an inflammatory dendritic cell population in rheumatoid arthritis. *Ann Rheum Dis* .

32. Piper C, Pesenacker AM, Bending D, Thirugnanabalan B, Varsani H, et al. (2014) T cell expression of granulocyte-macrophage colony-stimulating factor in juvenile arthritis is contingent upon Th17 plasticity. *Arthritis Rheumatol* 66: 1955-1960.
33. Shiomi A, Usui T, Ishikawa Y, Shimizu M, Murakami K, et al. (2014) GM-CSF but not IL-17 is critical for the development of severe interstitial lung disease in SKG mice. *J Immunol* 193: 849-859.
34. Kitching AR, Ru Huang X, Turner AL, Tipping PG, Dunn AR, et al. (2002) The requirement for granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor in leukocyte-mediated immune glomerular injury. *J Am Soc Nephrol* 13: 350-358.
35. Timoshanko JR, Kitching AR, Semple TJ, Holdsworth SR, Tipping PG (2005) Granulocyte macrophage colony-stimulating factor expression by both renal parenchymal and immune cells mediates murine crescentic glomerulonephritis. *J Am Soc Nephrol* 16: 2646-2656.
36. Haghighat A, Weiss D, Whalin MK, Cowan DP, Taylor WR (2007) Granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor exacerbate atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 115: 2049-2054.
37. Ditiatkovski M, Toh BH, Bobik A (2006) GM-CSF deficiency reduces macrophage PPAR-gamma expression and aggravates atherosclerosis in ApoE-deficient mice. *Arterioscler Thromb Vasc Biol* 26: 2337-2344.
38. Gaudreau S, Guindi C, Ménard M, Besin G, Dupuis G, et al. (2007) Granulocyte-macrophage colony-stimulating factor prevents diabetes development in NOD mice by inducing tolerogenic dendritic cells that sustain the suppressive function of CD4⁺CD25⁺ regulatory T cells. *J Immunol* 179: 3638-3647.
39. Gaudreau S, Guindi C, Ménard M, Benabdallah A, Dupuis G, et al. (2010) GM-CSF induces bone marrow precursors of NOD mice to skew into tolerogenic dendritic cells that protect against diabetes. *Cell Immunol* 265: 31-36.
40. Reed JA, Clegg DJ, Smith KB, Tolod-Richer EG, Matter EK, et al. (2005) GM-CSF action in the CNS decreases food intake and body weight. *J Clin Invest* 115: 3035-3044.
41. Sainathan SK, Hanna EM, Gong Q, Bishnupuri KS, Luo Q, et al. (2008) Granulocyte macrophage colony-stimulating factor ameliorates DSS-induced experimental colitis. *Inflamm Bowel Dis* 14: 88-99.
42. Däbritz J (2014) Granulocyte macrophage colony-stimulating factor and the intestinal innate immune cell homeostasis in Crohn's disease. *Am J Physiol Gastrointest Liver Physiol* 306: G455-465.
43. Däbritz J, Weinlage T, Varga G, Wirth T, Walscheid K, et al. (2015) Reprogramming of monocytes by GM-CSF contributes to regulatory immune functions during intestinal inflammation. *J Immunol* 194: 2424-2438.
44. Cornish AL, Campbell IK, McKenzie BS, Chatfield S, Wicks IP (2009) G-CSF and GM-CSF as therapeutic targets in rheumatoid arthritis. *Nat Rev Rheumatol* 5: 554-559.
45. Fraser JD, Weiss A (1992) Regulation of T-cell lymphokine gene transcription by the accessory molecule CD28. *Mol Cell Biol* 12: 4357-4363.
46. Shannon MF, Coles LS, Vadas MA, Cockerill PN (1997) Signals for activation of the GM-CSF promoter and enhancer in T cells. *Crit Rev Immunol* 17: 301-323.
47. Himes SR, Coles LS, Reeves R, Shannon MF (1996) High mobility group protein I(Y) is required for function and for c-Rel binding to CD28 response elements within the GM-CSF and IL-2 promoters. *Immunity* 5: 479-489.
48. Shang C, Attema J, Cakouros D, Cockerill PN, Shannon MF (1999) Nuclear factor of activated T cells contributes to the function of the CD28 response region of the granulocyte macrophage-colony stimulating factor promoter. *Int Immunol* 11: 1945-1956.
49. Cakouros D, Cockerill PN, Bert AG, Mital R, Roberts DC, et al. (2001) A NF-kappa B/Sp1 region is essential for chromatin remodeling and correct transcription of a human granulocyte-macrophage colony-stimulating factor transgene. *J Immunol* 167: 302-310.
50. Holloway AF, Rao S, Chen X, Shannon MF (2003) Changes in chromatin accessibility across the GM-CSF promoter upon T cell activation are dependent on nuclear factor kappaB proteins. *J Exp Med* 197: 413-423.
51. Jenkins F, Cockerill PN, Bohmann D, Shannon MF (1995) Multiple signals are required for function of the human granulocyte-macrophage colony-stimulating factor gene promoter in T cells. *J Immunol* 155: 1240-1251.
52. Thomas RS, Tymms MJ, McKinlay LH, Shannon MF, Seth A, et al. (1997) ETS1, NFkappaB and AP1 synergistically transactivate the human GM-CSF promoter. *Oncogene* 14: 2845-2855.
53. Brettingham-Moore KH, Rao S, Juelich T, Shannon MF, Holloway AF (2005) GM-CSF promoter chromatin remodelling and gene transcription display distinct signal and transcription factor requirements. *Nucleic Acids Res* 33: 225-234.
54. Cockerill PN, Bert AG, Jenkins F, Ryan GR, Shannon MF, et al. (1995) Human granulocyte-macrophage colony-stimulating factor enhancer function is associated with cooperative interactions between AP-1 and NFATp/c. *Mol Cell Biol* 15: 2071-2079.
55. Cockerill PN, Shannon MF, Bert AG, Ryan GR, Vadas MA (1993) The granulocyte-macrophage colony-stimulating factor/interleukin 3 locus is regulated by an inducible cyclosporin A-sensitive enhancer. *Proc Natl Acad Sci U S A* 90: 2466-2470.
56. Cockerill PN, Bert AG, Roberts D, Vadas MA (1999) The human granulocyte-macrophage colony-stimulating factor gene is autonomously regulated in vivo by an inducible tissue-specific enhancer. *Proc Natl Acad Sci U S A* 96: 15097-15102.
57. Johnson BV, Bert AG, Ryan GR, Condina A, Cockerill PN (2004) Granulocyte-macrophage colony-stimulating factor enhancer activation requires cooperation between NFAT and AP-1 elements and is associated with extensive nucleosome reorganization. *Mol Cell Biol* 24: 7914-7930.
58. Bert AG, Johnson BV, Baxter EW, Cockerill PN (2007) A modular enhancer is differentially regulated by GATA and NFAT elements that direct different tissue-specific patterns of nucleosome positioning and inducible chromatin remodeling. *Mol Cell Biol* 27: 2870-2885.
59. Smith PJ, Cousins DJ, Jee YK, Staynov DZ, Lee TH, et al. (2001) Suppression of granulocyte-macrophage colony-stimulating factor expression by glucocorticoids involves inhibition of enhancer function by the glucocorticoid receptor binding to composite NF-AT/activator protein-1 elements. *J Immunol* 167: 2502-2510.
60. Mirabella F, Baxter EW, Boissinot M, James SR, Cockerill PN (2010) The human IL-3/granulocyte-macrophage colony-stimulating factor locus is epigenetically silent in immature thymocytes and is progressively activated during T cell development. *J Immunol* 184: 3043-3054.
61. Lukens JR, Barr MJ, Chaplin DD, Chi H, Kanneganti TD (2012) Inflammasome-derived IL-1 β regulates the production of GM-CSF by CD4⁽⁺⁾ T cells and $\gamma\delta$ T cells. *J Immunol* 188: 3107-3115.