

## Editorial

# Interleukin-6, A Grown-Up Cytokine in EAE

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## Editorial

Three decades after its discovery by Kishimoto, et al [1], and this cytokine has been mentioned in more than 100.000 articles, 10.000 only in the last year, proving that it hasn't lost any piece of interest. Interleukin 6 (IL-6) is a cytokine so pleiotropic that it has caused confusion to researchers since the very beginning. Tadimitsu Kishimoto originally characterized it in 1985 as B-cell differentiation Factor (BSF-2) [1] after speculating that T cells must produce certain factors that induce growth and differentiation of B cells into antibody-producing cells [2]. Other labs were studying what was presumed unrelated growth factors at the time. These factors had several names and functions, interferon  $\beta$  [3,4], hybridoma growth factor [5], hepatocyte-stimulatory factor [6], cytotoxic T-cell differentiation factor [7],  $\beta$ -fibrinogen [8], amyloid protein, haptoglobin and hemopexin, to name a few [9]. When IL-6 was cloned, it was clear that these seemingly different factors were the same molecule, highlighting many different IL-6 activities, not limited to B cell immunology. It is now considered a highly multifunctional cytokine involved in the regulation of the immune response, inflammation, hematopoiesis, regeneration, metabolism, endocrine and nervous system [10].

A special feature of IL-6 signaling is the existence of two different signaling pathways. In the classic one, IL-6 binds to its membrane bound IL-6 Receptor (mIL-6R) which has a transmembrane and intracellular region but its binding does not lead to signaling. The complex of IL-6 and IL-6R associates with a second protein, gp130, which thereafter dimerizes and initiates intracellular signaling [11]. IL-6R presence is restricted to few cell types including hepatocytes, leukocytes [12] and in some brain regions. Cells, which do not express IL-6R, cannot respond to the cytokine *via* this pathway. However, in the more recently discovered trans-signaling pathway, IL-6 binds to a soluble form of the Receptor (sIL-6R), containing only the extracellular region and the complex binds to gp130 signaling subunits. As gp130 is ubiquitously expressed, trans-signaling confers IL-6 responsiveness to virtually all cells in the body, even the ones lacking IL-6R [13]. The sharing of gp130 partly explains the redundancy of the actions of these cytokines.

IL-6 plays a major role in the transition from innate immunity to acquired immunity [14] and it is implicated in the pathogenesis of numerous autoimmune disorders in humans, including Multiple Sclerosis (MS) [15,16]. Experimental Autoimmune Encephalomyelitis

(EAE) is a well-known animal model of MS; it is induced directly by administration of a myelin antigen, such as Myelin Oligodendrocyte Glycoprotein (MOG), together with an adjuvant. A critical role of IL-6 in the animal model of MS, EAE, has been demonstrated by a number of studies: systemic IL-6 KO mice are resistant to EAE [17-21], and neutralization of IL-6 with antibodies leads to a reduced disease [22], in line with the blockade of IL-6 trans-signaling, which delays the onset of adoptively transferred EAE [23]. Oppositely, some studies show that the virus-mediated transgenic expression of IL-6 in the CNS reduces EAE [24] and that the systemic administration of IL-6 also reduces the symptomatology in a viral model of EAE [25]. Therefore, IL-6 has a dual role in EAE, both potentiating and inhibiting it, reflecting the system's complexity. The next logical step at that point was to elucidate the identity of the key cell types that produce and respond to IL-6 in the CNS and whether the critical actions of IL-6 are peripheral or central, as it is produced from both peripheral and central sources. Establishing the specific contribution of each source of IL-6 to the development of the disease has been one of the top priorities of many investigation groups.

Hidalgo, et al. demonstrated that mice with astrocyte-driven IL-6 production did not develop classical EAE with leukocyte infiltration and demyelination of the spinal cord, but instead, they redirected the response to the cerebellum, suffering severe ataxia while mice expressing only astrocyte-driven IL-6 production (GFAP-IL6-IL-6 KO mice) were not resistant to EAE like total IL-6 KO, instead, they developed the same cerebellar EAE symptomatology as GFAP-IL6 mice [21]. Additionally, adoptive transfer of Tcell lines from IL-6<sup>+/+</sup> mice induced EAE in the mice with the intact IL-6 gene but less in the IL-6 KO mice, indicating that not only the encephalitogenic Tcells, but also local IL-6 in the brain, mediates the disease [18]. As astrocytic IL-6 have a key role in neuroinflammation [26,27] and astrocytes are the most abundant glial cell in the CNS, astrocytic IL-6 is an ideal candidate to be assessed. Mice lacking astrocytic IL-6 (Ast-IL-6 KO mice) exhibit a number of altered behaviors under normal (basal) conditions, including changes in activity, anxiety and learning [28,29]. It has been recently demonstrated that in contrast to results in total IL-6 KO mice, astrocytic-specific IL-6 deficiency is unable to prevent typical signs of EAE induction and has no prominent neuropathological effects. However, a delay in the onset of clinical signs was observed in Ast-IL-6 KO females, with fewer inflammatory infiltrates, decreased demyelination and some alterations in gliosis and vasogenesis compared to controls [30].

Majority of work has been done to characterize the role of local IL-6 in EAE but the CNS response to IL-6 is another critical issue to assess. Although trans-signaling appears to have a key role in EAE induction [23] it is extremely difficult to restrict the study only to central trans-signaling. However, mice with simultaneous astrocyte-driven production of both IL-6 and sgp130 (specific inhibitor of IL-6 and sIL-6R complex and thus the trans-signaling pathway) are expected to be a promising candidate to elucidate central trans-signaling role in EAE, as this model has already demonstrated to

counteract astrocyte-driven IL-6 in basal situations [27]. Regarding the role of IL-6 classic pathway in CNS, different cell populations must be studied, animals with lack of astrocyte-derived IL-6 membrane receptor have been created and initially characterized in basal conditions [27] and they might provide some answers when challenged with EAE to assess if they are capable of increasing clinical EAE's symptoms, as it has already been shown for immunized mice lacking cell surface expression of astrocyte-derived gp130 (GFAP-Cre gp130 KO); which not only developed chronic EAE, but also showed a significantly more severe symptomatology with worse recovery rate, mainly because of increased astrocyte apoptosis, increased numbers of CD4 T cells in the CNS and increased demyelination [31]. Increased severity is expected to be much more obvious in this GFAP-gp130 KO than in KO for specific cytoquines because lacking gp130 receptor impairs signal transducing of nearly all IL-6 cytokines family, not only IL-6, being therefore more difficult to compensate. As GFAP-Cre also affects a subpopulation of neurons, this group decided to delete gp130 in neurons to assess whether astrocytes or neurons were responsible for aggravation of EAE in GFAP-Cre gp130 KO mice, showing that only the absence of gp130 on astrocytes, but not on neurons, was the responsible for the increased susceptibility phenotype [31]. They further demonstrated that diminished activation of the gp130-SHP2/Ras/ERK pathway reproduced all pathological features observed in GFAP-Cre gp130 KO mice, including astrocyte loss, lack of Astrogliosis, a significantly more severe clinical course, increased T-cell infiltration, and severe demyelination; while mice with intact gp130-SHP2/Ras/ERK signaling but impaired STAT activation in astrocytes developed a similar clinical course compared to floxed controls and decreased compared to GFAP-Cre gp130 KO and GFAP-Cre gp130-SHP2/Ras/ERK KO, due to an astrocyte-dependent reduction of autoimmune t cells in the CNS [31].

IL-6 has also a major role in Th17 cell differentiation from naive CD4+ T-cells [32]; particularly in the EAE model [33,34]. Moreover, showing the importance of infiltrating cells in EAE pathology, EAE-resistant IL-6 KO mice demonstrated a deficiency in Th17 cells infiltrated in the CNS [35]. When responsiveness to IL-6 is eliminated only in T helper cells there is resistance to EAE, as IL-21 pathway is intact but not active in the absence of IL-6 [36]. Th17 cells produce IL-17 (among other cytokines) which enhances IL-6 production by astrocytes, which in turn induces differentiation of Th17, cells in a positive feedback loop between IL-17 and IL-6 *via* activation of NF- $\kappa$ B and STAT-3 [37]. As Ast-IL-6 KO mice are not resistant to EAE induction, finally reaching the same score as WT, one can venture to say that astrocyte-derived loop is not necessary for the development of the disease as it is probable that neuronal, endothelial and microglial IL-6 allow this positive feed-back between IL-17 and IL-6. Now the efforts are directed to assess the role of IL-6 expression from other brain populations and to further characterized the inflammatory response in these models. After thirty years of study, the relevance of IL-6 is still growing.

## References

- Hirano T, Taga T, Nakano N, Yasukawa K, Kashiwamura S, Shimizu K, et al. Purification to homogeneity and characterization of human B-cell differentiation factor [BCDF or BSFp-2]. *Proceedings of the National Academy of Sciences*. 1985; 82: 5490–5494.
- Kishimoto T. Factors affecting B-cell growth and differentiation. *Annual Review of Immunology*. 1985; 3: 133–157.
- Weissenbach J, Chernajovsky Y, Zeevi M, Shulman L, Soreq H, Nir U, et al. Two interferon mRNAs in human fibroblasts: *in vitro* translation and Escherichia coli cloning studies. *Proceedings of the National Academy of Sciences*. 1980; 77: 7152–7156.
- Zilberstein A, Ruggieri R, Korn J, Revel M. Structure and expression of cDNA and genes for human interferon-beta-2, a distinct species inducible by growth-stimulatory cytokines. *The EMBO Journal*. 1986; 10: 2529.
- Brakenhoff J, De Groot E, Evers R, Pannekoek H, Aarden L. Molecular cloning and expression of hybridoma growth factor in Escherichia coli. *The Journal of Immunology*. 1987; 139: 4116–4121.
- Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon beta 2/B-cell stimulatory factor type 2 shares identity with monocyte-derived hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proceedings of the National Academy of Sciences*. 1987; 84: 7251–7255.
- Takai Y, Wong G, Clark S, Burakoff S, Herrmann S. B cell stimulatory factor-2 is involved in the differentiation of cytotoxic T lymphocytes. *The Journal of Immunology*. 1988; 140: 508–512.
- Andus T, Geiger T, Hirano T, Northoff H, Ganter U, Bauer J, et al. Recombinant human B cell stimulatory factor 2 (BSF-2/IFN-b2) regulates b-fibrinogen and albumin mRNA levels in Fao-9 cells. *FEBS letters*. 1987; 221: 18–22.
- Kishimoto T. IL-6: from its discovery to clinical applications. *International Immunology*. 2010; 22: 347–352.
- Kishimoto T, Akira S, Narazaki M, Taga T. Interleukin-6 family of cytokines and gp130. *Blood*. 1995; 86: 1243–1254.
- Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp130. *Cell*. 1990; 63: 1149–1157.
- Taga T. IL6 signalling through IL6 receptor and receptor-associated signal transducer, gp130. *Research in Immunology*. 1992; 143: 737 – 739.
- Jones SA, Horiuchi S, Topley N, Yamamoto N, Fuller GM. The soluble interleukin 6 receptor: mechanisms of production and implications in disease. *The FASEB Journal*. 2001; 15: 43–58.
- Jones SA. Directing transition from innate to acquired immunity: defining a role for IL-6. *The Journal of Immunology*. 2005; 175: 3463–3468.
- Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, et al. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nature Medicine*. 2002; 8: 500–508.
- Mycko MP, Papoian R, Boschert U, Raine CS, Selmaj KW. cDNA microarray analysis in multiple sclerosis lesions: Detection of genes associated with disease activity. *Brain*. 2003; 126: 1048–1057.
- Eugster HP, Frei K, Kopf M, Lassmann H, Fontana A. IL-6 deficient mice resist myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. *European Journal of Immunology*. 1998; 28: 2178–2187.
- Mendel I, Katz A, Kozak N, BenNun A, Revel M. Interleukin-6 functions in autoimmune encephalomyelitis: A study in gene-targeted mice. *European Journal of Immunology*. 1998; 28: 1727–1737.
- Okuda Y, Sakoda S, Bernard CC, Fujimura H, Saeki Y, Kishimoto, T, et al. IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein. *International Immunology*. 1998; 10: 703–708.
- Samoilova EB, Horton JL, Hilliard B, Liu TS, Chen Y. IL-6-deficient mice are resistant to experimental autoimmune encephalomyelitis: Roles of IL-6 in the activation and differentiation of autoreactive T cells. *Journal of Immunology*. 1998; 161: 6480–6486.
- Giralt M, Ramos R, Quintana A, Ferrer B, Erta M, CastroFreire M, et al. Induction of atypical EAE mediated by transgenic production of IL-6 in astrocytes in the absence of systemic IL-6. *Glia*. 2013; 61: 587–600.
- Gijbels K, Brocke S, Abrams JS, Steinman L. Administration of neutralizing antibodies to interleukin-6 (IL-6) reduces experimental autoimmune encephalomyelitis and is associated with elevated levels of IL-6 bioactivity

- in central nervous system and circulation. *Molecular Medicine*. 1995; 1: 795–805.
23. Linker RA, Luhder F, Kallen KJ, Lee DH, Engelhardt B, Rose-John S, et al. IL-6 transsignaling modulates the early effector phase of EAE and targets the blood-brain barrier. *Journal of Neuroimmunology*. 2008; 205: 64–72.
24. Willenborg DO, Fordham SA, Cowden WB, Ramshaw IA. Cytokines and murine autoimmune encephalomyelitis: Inhibition or enhancement of disease with antibodies to select cytokines, or by delivery of exogenous cytokines using a recombinant vaccinia virus system. *Scandinavian Journal of Immunology*. 1995; 41: 31–41.
25. Rodriguez M, Pavelko KD, McKinne CW, Leibowitz JL. Recombinant human IL-6 suppresses demyelination in a viral model of multiple sclerosis. *Journal of Immunology*. 1994; 153: 3811–3821.
26. Campbell IL, Abraham CR, Masliah E, Kemper P, Inglis JD, Oldstone MB, et al. Neurologic disease in transgenic mice by cerebral overexpression of interleukin 6. *Proceedings of the National Academy of Sciences USA*. 1993; 90: 10061–10065.
27. Campbell IL, Erta M, Lim SL, Frausto R, May U, Rose-John, et al. Trans-signaling is a dominant mechanism for the pathogenic actions of interleukin-6 in the brain. *Journal of Neuroscience*. 2014; 34: 2503–2513.
28. Quintana A, Erta M, Ferrer B, Comes G, Giralt M, Hidalgo J. Astrocyte-specific deficiency of interleukin-6 and its receptor reveal specific roles in survival, body weight and behavior. *Brain, Behavior and Immunity*. 2013; 27: 162–173.
29. Erta M, Giralt M, Esposito FL, FernandezGayol O, Hidalgo J. Astrocytic IL-6 mediates locomotor activity, exploration, anxiety, learning and social behavior. *Hormones and Behavior*. 2015; 73: 64–74.
30. Erta M, Giralt M, Jimenez S, Molinero A, Comes G, Hidalgo J. Astrocytic IL-6 Influences the Clinical Symptoms of EAE in Mice. *Brain Sciences*. 2016; 6: 15.
31. Haroon F, Drogemuller K, Handel U, Brunn A, Reinhold D, Nishanth G, et al. Gp130-dependent astrocytic survival is critical for the control of autoimmune central nervous system inflammation. *The Journal of Immunology*. 2011; 186: 6521–6531.
32. Kimura A and Kishimoto T. IL-6: Regulator of Treg/Th17 balance. *European journal of immunology*. 2010; 40: 1830–1835.
33. Murphy AC, Lalor SJ. Infiltration of Th1 and Th17 cells and activation of microglia in the CNS during the course of experimental autoimmune encephalomyelitis. 2010; 24: 641–651.
34. Serada S, Fujimoto M, Mihara M, Koike N, Ohsugi Y, Nomura S, et al. IL-6 blockade inhibits the induction of myelin antigen-specific Th17 cells and Th1 cells in experimental autoimmune encephalomyelitis. *Proceedings of the National Academy of Sciences*. 2008; 105: 9041–9046.
35. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature*. 2006; 441: 235–238.
36. Korn T, Mitsdoerffer M, Croxford AL, Awasthi A, Dardalhon VA, Galileos G, et al. IL-6 controls Th17 immunity *in vivo* by inhibiting the conversion of conventional T cells into Foxp3+ regulatory T cells. *Proceedings of the National Academy of Sciences*. 2008; 105: 18460–18465.
37. Ma X, Reynolds SL, Baker BJ, Li X, Benveniste EN, Qin H. IL-17 enhancement of the IL-6 signaling cascade in astrocytes. *The Journal of Immunology*. 2010; 184: 4898–4906.