The role of IL-2, and IL-27, in combination with retinoic acid in the B cells, APC, CD4+ T cell subsets (Th1, Th17 and Treg), and CD8+ T cells response in EAE modele

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Abstract

Background:

Cytokines are critical players in the regulation of immune responses. With the help of these soluble factors, immune cells undergo proliferation, activation, differentiation, and inactivation or even cell death. Among various types of cells involved in immune responses, CD4 T cells play central roles in immune regulation. Immune-suppressive function and Differentiation of these T and B cell populations by Tregs, and B regs, cells is affected by various cytokines. These Tregs, and B regs, microenvironment interact in the process and context of Autoimmunity.

Objective:

To determine the effective potential of IL-2 and IL-27 in combinations with retinoic acid on maintaining tolerance and balance modification of B cells, APC, CD4+T cell subsets (Th1, Th17 and Treg), and CD8+ T cells response in EAE model

Methods:

After EAE induction, in the presence of IL-2 in combination with retinoic acid, IL-27 in combination with retinoic acid on the 10 weeks old female C57BL/6 mice, we will be analyze the spleenocyte levels to determining Tbet, RORγt, FOXP3, IFN γ, IL-17, IL-10, IL-1β, TNFα, IL-12, IL-6 and HPRT1, gene expressions using Real time PCR method. Will perform according to the protocol of the manufacturer

Lymphocyte Subsets (Th1, Th17, Treg, and B cells), will be analysis from spleenocyte, using the technique Flow Cytometer.

Results/ Conclusions:

IL-2 and IL-27 in-combination with retinoic acid exerts a dual effect (inhibition versus enhancement) in maintaining tolerance and balance modifying in B cells, APC, CD4+T cell subsets (Th1, Th17 and Treg), and CD8+ T cells. Which are implicated in the immune-pathogenesis, molecular mechanism, and cytokine pathways, suggest that IL-2 and IL-27 in-combination with retinoic acid both prevention and therapeutically relevant in Multiple autoimmunity, multiple Cancer, and graft rejection. However, while exciting discoveries have been made, further work is required to understand the diverse roles of IL-2 and IL-27 in-combination with retinoic acid and in maintaining tolerance and balance modifying in B cells, APC, CD4+T cell subsets (Th1, Th17 and Treg), and CD8+ T cells.

Key Word: Immunotherapy; C57BL/6 mice; EAE; B cells; APC; CD4+ T cell Subsets (Th1, Th17, and Treg); and CD8+ T cells

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1. Introduction

IL-2 is a single polypeptide of molecular weight 15.5 kDa, 133 amino acid residues long. There is only a single IL-2 gene locus in humans, on chromosome 4. IL-2 is a globular protein containing two sets of a-helical domains, lying at right angles to each other (1).These α-helical regions are involved in the binding to the receptor, and indeed this helical motif is found in many other cytokines, involved in binding to their respective receptors. IL-2 is required for the survival and expansion of T reg cells; T reg cells from IL-2– deicient donors fail to survive in IL-2-/- hosts (2), or to expand in the Asence of IL-2R signals (2-4). Blocking IL-2R(5), or neutralizing IL-2 (6), reduces T reg cell numbers. IL-2 also plays a role in the stability of FOXP3 expression and FOXP3-dependent gene signature (4, 7, 8). Although these studies demonstrated that IL-2 is an essential resource for T reg cells, the mechanisms regulating the critical cell source providing IL-2 remained to be identified.

In immune cells, IL-27 is mainly produced from antigen-presenting cells (APCs). The mRNA expressions of IL-27p28 and Ebi3 are induced in APCs by Toll-like receptor (TLR) stimulation, such as lipopolysaccharide (LPS), CpG, and Poly(I:C). Upon IL-27p28 production, nuclear factor (NF)-κB is important in early induction phase mediated by TLR. In addition, IFN-γ and IFN-α/β can amplify IL-27p28 expression by activating IFN response fragment-1 (IRF-1) and IRF-8 (9). Myeloid differentiation factor (MyD88)-independent Toll/IL-1R-related domain-containing adaptor-inducing IFN (TRIF) mediated activation of IRF-3 and IRF-4 is also related to efficient IL-27p28 and Ebi3 production (10) . IL-27 is able to block IL-17 secretion from effector CD4+ or CD8+ T cells isolated from the central nervous system (CNS) of infected mice (11).

The effect of atRA on Treg and Th17 cells is dependent upon the RA receptor/retinoid X receptor heterodimer (12) (13). Because the pathogenesis and development of many autoimmune diseases is affected by the imbalance between Treg and Th17 cells, the role of atRA in regulating this balance may greatly affect the progress of autoimmune diseases. The orphan nuclear receptor, RORct, has been implicated in the gene transcription of Th17 cells. TGF-b induces high levels of RORct and further

promotes Th17 cell development in the presence of IL-6. However, the addition of atRA to cultures containing TGF-b and IL-6 greatly reduces RORct expression and Th17 cell differentiation (14)

2. Immuno-pathogenesis of Multiple autoimmunity

Any damage to CNS tissue leads to the activation of CNS resident immune cells in particular, microglial cells, which up-regulate Major Histocompatibility Complex (MHC, also known as HLA in humans) and Co-stimulatory molecules. These cells start to release Cytokines and Chemokine, thereby paving the way for the entry of monocytes, lymphocytes and cells with a phenotype similar to Dendritic cells into the lesion. Microglial cells are important for generating and maintaining the inflammatory milieu, whereas dendritic cells seem to play a central role in antigen presentation to invading T cells (15),(16).

The pathogenic role of Th1 and Th17 cells, CD4+FoxP3+ regulatory T cells (Tregs) have been shown to down-regulate the immune response and have been shown to play a critical in preventing generalized multi organ autoimmunity (17),(18). Moreover, MS patients possess either a lower frequency of Tregs or impairment in their suppressor function, which promotes disease development (19). Thus, expansion of Tregs in vivo has the potential to treat autoimmune disease as well as prevent the rejection of organ transplants (20). While most T cells express IL-2 receptors (IL-2Rs) upon activation, Tregs express IL-2Rs constitutively and dependent on IL-2 for their growth and survival (21).

IL-27 (an IL12/IL23 family member) is a negative regulator of Th17 cell differentiation and can prevent inflammatory demyelination in the EAE model (22). IL-27 drives the expansion and differentiation of IL-10-producing Tregs by inducing the expression of three key molecules: the basic leucine-zipper transcription factor Maf (generally known as c-Maf), the IL-21, and ICOS (an inducible T-cell costimulator structurally and functionally related to CD28. Moreover, IL-27-driven c-MAF expression trans activates the production of IL-21, which favor the expansion of IL-27-induced Tr1 cells. ICOS also promotes IL-27-driven Tregs. Each of these elements is essential, because the loss of c-MAF, IL-21 signaling, or ICOS reduces the frequency of IL-27-induced Treg differentiation (23). Exacerbation of EAE was demonstrated in IL-27-deficient mice, and interestingly, IL-27 treated mice had markedly reduced CNS inflammatory infiltration, indicating the down regulation of Th17 phenomena (24).

In parallel with the initial discovery that RA enhanced iTreg cell differentiation, RA was observed to suppress Th17 cell generation (25). RA was shown to inhibit IL-6R and IL-23R upregulation induced by TGF-β and IL-6, respectively (26),(27). In addition to Th17 cells, RA can exert direct regulatory effects on other effector T cell populations (28). For instance, RA was shown to inhibit IFN- γ production from CD8+ T cells and Th1 cells (29),(30). In serum free cultures, RA was observed to dramatically enhance TCR mediated CD4+ T cell proliferation in an IL-2 dependent manner (31). The NFAT family of transcription factors regulates an array of functions in multiple cell types; in T cells these include production of IL-2 and the full acquisition of effector properties (32),(33).

Precisely how RA/RARα signals mediate early T cell activation events is unclear. RARα is potent transcriptional regulator of gene networks and known to constitutively bind to DNA. Such binding may exert a tonic influence on the DNA binding capacity of other proteins involved in the regulation of T cell activation.

3. Molecular biology of IL-2 and its receptor

IL-2 is a single polypeptide of molecular weight 15.5 kDa, 133 amino acid residues long. There is only a single IL-2 gene locus in humans, on chromosome 4. IL-2 is a globular protein containing two sets of a-helical domains, lying at right angles to each other (34) These α-helical regions are involved in the binding to the receptor, and indeed this helical motif is found in many other cytokines, involved in binding to their respective receptors. Il-2 is only produced by activated T-cells, especially the CD4q T-helper cell population, although CD8q T-cytotoxic cells can be stimulated in vitro to produce Il-2 (35).

It is a potent immunomodulator, and has an important role in both the activation and maintenance of an immune response and in lymphocyte development. Interleukin-2 serves to activate numerous key cells in the immune system, including helper T cells, cytotoxic T cells, B lymphocytes, natural killer cells, tumour infiltrating lymphocytes and macrophage-monocyte cells (36).Two other components of the receptor have been identified: the β and γ chains (37),(38). IL-2 in fact binds to α receptor complex made up of three chains. The individual chains have low affinities for IL-2, but when combined they act as a high-affinity complex. The α subunit on its own represents the low affinity state, the β,γ complex has an intermediate affinity, and the high affinity complex contains the α,β,γ chains and is the active receptor (39). Cells expressing only β,γ chains can be stimulated, but only at very high concentrations of IL-2.The ligand is thought to bind to the α and β subunits first, followed by heterodimerization of the α,β,γ chains to activate the intracellular downstream signalling mechanisms. The α subunit gene has been identified and is located on chromosome 10 in the human (40). The protein product of this gene is a 251 amino acid chain, with a long extracellular domain responsible for binding IL-2, α 19 amino acid hydrophobic region postulated to be the transmembrane domain, and a C-terminal intracellular domain of only 13 amino acids (41). This intracellular domain is too short to act as an important site for signal transduction, and lacks any known consensus sequence for intracellular signalling. Cellular expression of the α subunit is tightly regulated. The gene has three important regions located either upstream or downstream of the transcribed region, which act as enhancers of transcription. One of these sites is called PRRI (positive regulatory region I): it binds NF-kB and so in activated T-cells, the NF-kB produced acts to stimulate α-subunit production (41). Another site, PRRIII, can bind a transcription factor called Stat 5, which is itself upregulated during IL-2 stimulation, providing a positive feedback mechanism (41). The b chain is a 70–75 kDa protein located on chromosome 22 (42). This type 1 membrane protein has an intracellular domain of 286 amino acid residues that contains two key signaling elements: Box 1 and Box 2. It is constitutively expressed on resting lymphocytes, monocytes/ macrophages and neutrophils, and is upregulated upon T-cell activation. γ chain is again a type 1 membrane protein and is 347 amino acids in size giving a molecular weight of 64 kDa (38). The genetic locus is on the X chromosome (43). It has a long intracellular domain that is vital for IL-2 signalling, and is constitutively expressed on lymphocytes, monocytes and neutrophils. Both the b and c chains are related to the cytokine receptor superfamily type 1, unlike the α subunit. These receptors are characterized by the possession of similar structural motifs: for example, four conserved cysteine residues at the N-terminus. Of significance is the fact that the b and c subunits are constituents of other interleukin receptors. The c chain acts in at least six other cytokine receptors and has been called the common receptor cc (43).

4. Molecular characterization of IL-27R

IL-27 is a heterodimeric cytokine composed of two subunits: EBI3 and p28 (44). EBI3 is a 34-kDa glycoprotein similar to the p40 subunits of IL-12 (45). The EBI3 reversibly binds to IL-12p35-related subunit, namely p28, to form heterodimeric cytokine IL-27 (45),(46). The connection between p28 and EBI3 is labile and these subunits can be secreted independently (44). EBI3 is also capable of binding to the IL-12p35 to form IL-35 (47). The human p28 gene encodes a 24.5kDa polypeptide (44). The p28 is structurally similar to an IL-6/IL-12 family, composed of a long chain of four α helix bundle named A–D

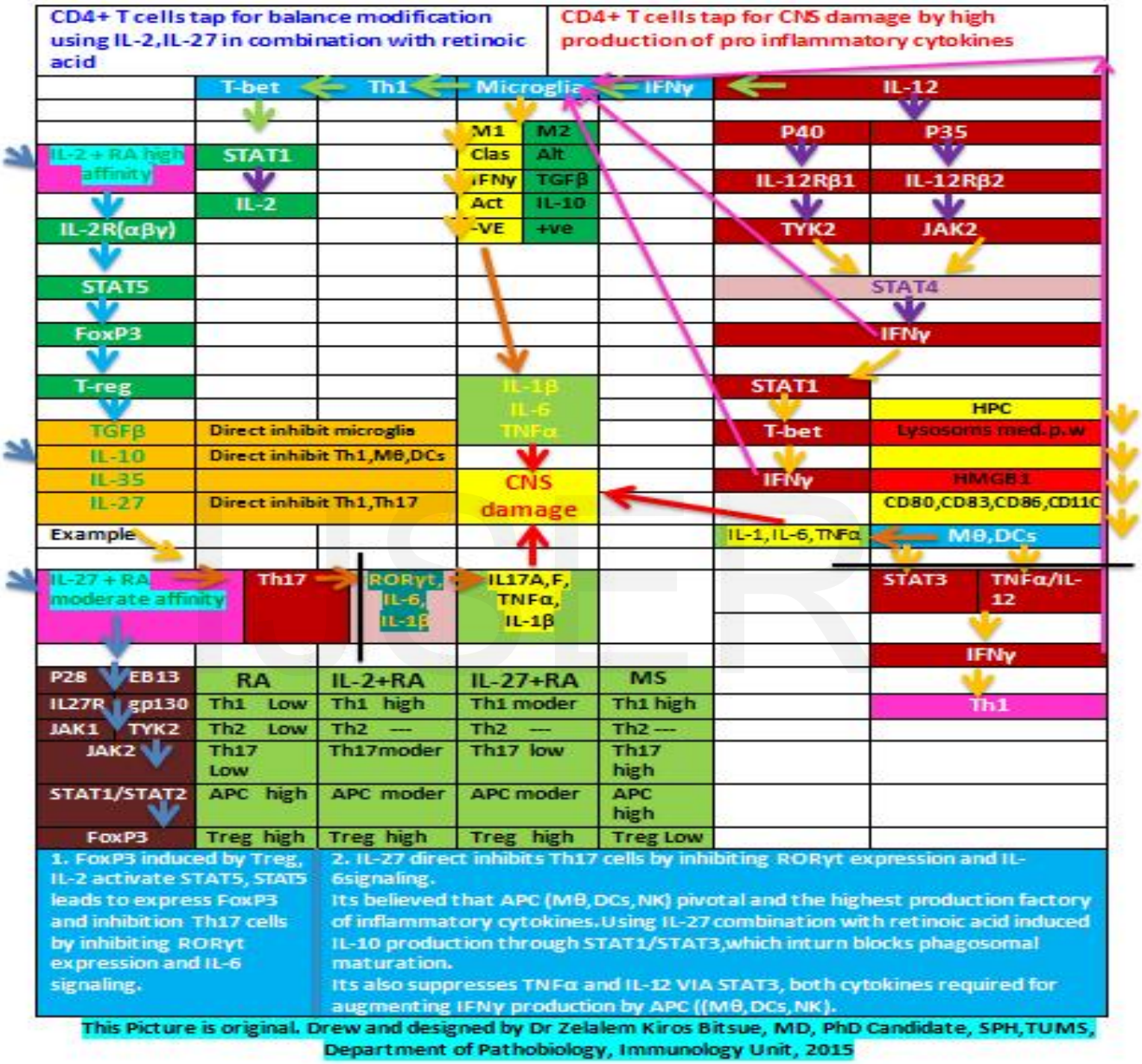
from the N terminus to the C terminus (48). The polypeptide loop connecting the p28 C and D α helices contains a stretch of polyglutamic acids (poly-E) unique among helical cytokines and is highly conserved (49). The p28 alone can suppress IL-27 mediated Th1 responses (50), and IL-6 mediated signaling (51). IL-27 is mainly produced by activating APCs such as DCs and macrophages. Macrophages-stimulated with TLRs agonists, (polyinosinic: polycytidylic acid (poly (I:C)), Lipopolysaccharide (LPS), or R848 can induce both subunits of IL-27(52). It has been reported that p28 production is completely dependent on the TLR4-associated myeloid differentiation factor 88 (MyD88) mediated pathway and partially dependent on NF-κBc-Rel transcription factor (53). MyD88 also regulates p28 expression through binding of AP-1/c-Fos to the p28 promoter in both human and mouse macrophages. However, the binding of c-Fos to the p28 promoter can be blocked by overexpression of p38 MAPK (54). In addition, TLR4 can induce the expression of p28 subunit through activating the TIR domain-containing adaptor inducing IFN-β (TRIF) and IFN regulatory factor 3 (IRF3) pathways (55). TLR2, TLR4, and TLR9-associated MyD88 are required for the induction of EBI3 expression through binding of NF-kB subunits (p50/p65) and PU.1 to the EBI3 promoter(56).

5. Molecular Biology of Retinoid

Retinoids are natural and synthetic derivatives of vitamin A (retinol) (57). Vitamin A is an essential vitamin that must be derived from the diet through the ingestion of vitamin A–rich foods as well as foods containing the carotenoid b-carotene, which is composed of 2 molecules of retinol. atRA plays a vital role in normal embryogenesis21 and in such postnatal processes as skin and epithelial homeostasis, hematopoiesis, and spermatogenesis (58). An important early discovery was the demonstration that atRA could promote cellular differentiation in vitro (59),(60). These findings led to the use of atRA and other natural and synthetic retinoids for the treatment of cancer (61),(62). Today, atRA is standard therapy for the management of acute promyelocytic leukemia(63), and is in various phases of clinical trials for a number of other hematological and solid tumors (58). The common mechanisms underlying cancer and cardiovascular diseases (ie, perturbations in differentiation and growth) suggest that retinoids could also be of therapeutic value in the treatment of certain vascular diseases atRA is a small lipophilic molecule (300 daltons) that circulates in plasma bound to albumin at a concentration of 1 to 10 nmol/L (64),(65). Experimental and clinical pharma-cokinetic studies show peak plasma levels of atRA occurring 2 hours after oral administration and near-complete plasma clearance after 6 hours (66). Importantly, therapeutic levels of atRA in humans approach '1 mmol/L, which is the standard concentration used for most in vitro studies. At the cellular level, atRA traverses the plasma membrane owing to its lipophilic structure and then interacts with 1 of 2 cellular retinoic acid (RA) binding proteins (CRABPs). CRABP I is widely expressed and appears to function as an intracellular buffer facilitating atRA metabolism to more polar retinoids via key cytochrome P-450 isozymes (67). A second less understood fate of intracellular atRA is its isomerization to 9-*cis* RA and 13-*cis* RA. The physiological function of 13-*cis* RA is unclear, although its half-life is considerably longer than that of atRA (13 hours versus 1 to 2 hours), it is less toxic than atRA, and it can readily undergo isomerization to atRA (66). 9-*cis* RA can also isomerize to atRA (and vice versa), but, because circulating levels of 9-*cis* RA are much lower than those of atRA, the physiological significance of this isomerization is difficult to ascertain. Clinical trials using 13-*cis* RA and 9-*cis* RA have shown promising results in reducing the incidence of secondary head and neck tumors32 and acute promyelocytic leukemia (67), respectively. A third fate of intracellular retinoids is nuclear translocation and binding to the retinoid receptors (68). The RA receptors (RARs a, b, and g) bind both atRA and 9-*cis* RA, whereas the retinoid X receptors (RXRs a, b, and g) bind 9-*cis* RA. 13-*cis* RA is not a ligand for the retinoid receptors, but, as shown in the Figure, it can readily convert into a retinoid receptor ligand. In general, the RARs are expressed at higher levels than the RXRs, which often require sensitive methods of detection such as reverse transcriptase–polymerase chain reaction. The mRNA expression of RARa, RXRa, and RXRb is ubiquitous, whereas RARb (central nervous system), RARg (skeletal muscle

precursors and skin and lung epithelia), and RXRg (skeletal muscle) exhibit tissue-restricted patterns of expression (69),(70),(71),(72).

6. Signaling pathways and transcription factor that regulate the differentiation of T-reg



7. Materials and Methods

Animals will house in specific pathogen-free conditions and housed in a temperature-controlled room under illumination with a 12 h light: 12 h dark cycle (lights on from 06h00 to 18h00) and both food and water.

After one week, EAE is induced in C57BL/6 mice by immunization with an emulsion of MOG35-55 in complete Freund's adjuvant (CFA), followed by administration of pertussis toxin (PTX) in PBS.

On day 10 after EAE induction recombinant proteins fused with all Trans Retinoic acid given ti each group

On day 21 after EAE induction, the spleens will be obtained from all groups of treated mice. Splenocyte will be obtained using cell stainer and prepare a single cell suspension, at least 2ml of spleenocyte

Then spleenocyte taken to Cell culture, after 72 hours culture, the supernatant will take to Flow Cytometer , ELISA, and RT-PCR.

Data will express as mean ± SEM. Statistical analyses will perform using Student's t test or two-factor NOVA as appropriate, with a P value of <0.05 consider to be statistically significant.

8. Conclusion

IL-2 and IL-27 in-combination with retinoic acid exerts a dual effect (inhibition versus enhancement) in maintaining tolerance and balance modifying in CD4+T cell subsets (Th1, Th17 and Treg), and CD8+ T cells. Which are implicated in the immunopathogenesis, molecular mechanism, and cytokine pathways, suggest that IL-2 and IL-27 in-combination with retinoic acid both prevention and therapeutically relevant in Multiple autoimmunity, multiple Cancer, and graft rejection. However, while exciting discoveries have been made, further work is required to understand the diverse roles of IL-2 and IL-27 in-combination with retinoic acid and in maintaining tolerance and balance modifying in T cell subsets (Th1, Th17 and Treg), and CD8+ T cells.

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