# Regulatory Mechanisms and Their Therapeutic Implications of IL-12/IL-4 Production in T Helper Cell Differentiation

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T helper lymphocytes can be divided into two distinct subsets of effector cells, Th1 and Th2 cells, based on their functional capabilities and the profile of cytokines they produce. The Th1 subset of $CD4^+$ T cells secretes cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α, and induces cell-mediated immune responses. The Th2 subset produces cytokines such as IL-4 and IL-5 that help B cells to proliferate and differentiate and is associated with humoral immune responses. Recent studies indicate that the ratio of these two Th cell types, Th1 and Th2, is closely correlated with the outcome of many diseases. Polarized Th1-type and Th2-type responses play different roles in protection, Th1 being effective in the defense against intracellular pathogens and Th2 against intestinal nematodes. Moreover, Th1 responses predominate in organ-specific autoimmune disorders, acute allograft rejection, unexplained recurrent abortions, and in some chronic inflammatory disorders of unknown etiology. In contrast, Th2 responses predominate in Omenn's syndrome, transplantation tolerance, chronic graft *versus* host disease, systemic sclerosis, and allergic diseases. The nature of Th1 or Th2 polarizing signals is not yet fully understood. However, the cytokines that are present in the environment of the $CD4^+$ T cell at the time it encounters the antigen importantly regulate the differentiation of Th cells into either Th1 or Th2 subsets.

Interleukin-12 (IL-12), an important part of the innate immune response, is a major cytokine that drives differentiation of T cells toward Th1 pathway and thus has a central role in the development and progression of the Th1-dominated diseases. The pathogenesis of cell-mediated autoimmune diseases, such as multiple sclerosis, uveitis, diabetes, arthritis, and others, is thought to be in a large measure driven by interferon-gamma-producing antigen-specific T cells polarized toward the Th1 phenotype. Therefore, pharmacological control of IL-12 production may be a key strategy in modulating specific immune-mediated diseases dominated by type-1 cytokine responses.

First, we have investigated the effect of auranofin (AF), an anti-rheumatic gold compound, on IL-12 production in mouse macrophages and dendritic cells, and studied whether AF-mediated inhibition of IL-12 production can regulate a cytokine profile of antigen (Ag)-primed $CD4^+$ Th cells. Treatment with AF significantly inhibited IL-12 production in lipopolysaccharide (LPS)-stimulated

macrophages and also in CD40L-stimulated dendritic cells. AF-pretreated macrophages reduced their ability to induce IFN-γ and increased the ability to induce IL-4 in Ag-primed $CD4^+$ T cells. AF did not influence the cell surface expression of the class II MHC molecule and the costimulatory molecules CD80 and CD86. Addition of recombinant IL-12 to cultures of AF-pretreated macrophages and $CD4^+$ T cells restored IFN-γ production in Ag-primed $CD4^+$ T cells. The in vivo administration of AF resulted in the inhibition of IL-12 production by macrophages stimulated in vitro with LPS or heat-killed Listeria monocytogenes (HKL), leading to the inhibition of Th1 cytokine profile (decreased IFN-γ and increased IL-4 production) in Ag-primed $CD4^+$ T cells. These findings may explain some known effects of AF including anti-rheumatic effects and the inhibition of encephalitogenicity, and point to a possible therapeutic use of AF in the Th1-mediated immune diseases such as autoimmune diseases.

Furthermore, as a way of regulating IL-12 production, the effects of several medicinal compounds on IL-12 production are described in mouse macrophages and their action mechanisms are also discussed at the molecular levels. For example, retinoids significantly inhibited IL-12 production in LPS-activated macrophages through direct interaction of nuclear factor-κB and retinoid X receptors. Importantly, retinoid-mediated inhibition of IL-12 production in macrophages suppressed Th1 cytokine profile in $CD4^+$ T cells. Other compounds including sulfasalazine showed similar pattern of regulation in IL-12 production and in cytokine profile of antigen-primed $CD4^+$ T cells. These studies suggest a possible therapeutic use of those compounds in Th1 cell-mediated autoimmune diseases including rheumatoid arthritis.

Allergic disorders affect at least 20% of the population in developed countries. They include hay fever, asthma, atopic dermatitis and food allergies. These signs are associated with high levels of serum IgE and allergen-specific IgE and eosinophilia. They are dependent upon IL-4 and IL-5 released from allergen-specific CD4 T cells expressing the T helper type 2 (Th2) cytokine profile. IL-4 is a pleiotropic cytokine that modulates the differentiation and the biologic activities of virtually all cells of haematopoietic origin. It plays a central role in Th2-type immune responses, such as IgE production and immediate allergic inflammation. It may be involved in the exacerbation of allergic diseases.

We previously found that several endocrine disruptors including BPA and NP significantly enhanced IL-4 production in KLH-primed $CD4^+$ T cells in a concentration-dependent manner. Treatment with BPA or NP in vivo resulted in significant increase of IL-4 production in $CD4^+$ T cells and of antigen-specific IgE levels in the sera of KLH-primed mice. Furthermore, BPA and NP enhanced the activation of IL-4 gene promoter in EL4 T cells transiently transfected with IL-4 promoter/reporter constructs, and the enhancing effect mapped to a region in the IL-4 promoter containing binding sites for NF-AT. Activation of T-lymphocytes by PMA/ionomycin resulted in markedly enhanced binding activities to the NF-AT site, which significantly increased upon addition

of BPA or NP, as demonstrated by the electrophoretic mobility shift assay, indicating that the transcription factor NF-AT was involved in the enhancing effect of BPA and NP on IL-4 production. The enhancement of IL-4 production by BPA or NP was significantly reduced by nitrendipine, a blocker of $Ca^{2+}$ influx, and by FK506, a calcineurin inhibitor. FK506 inhibited the NF-AT-DNA binding activity and IL-4 gene promoter activity enhanced by BPA or NP. These results represent the first report describing possible enhancement of allergic response by EDs through increasing IL-4 production in $CD4^+$ T cells and antigen-specific IgE levels in the sera via the stimulation of $Ca^{2+}$/calcineurin-dependent NF-AT activation.

Recently we have investigated the inhibitory activity of IL-4 production in activated T cells by screening ceramide derivatives prepared by solid phase combinatorial chemistry. Many ceramide derivatives significantly inhibited IL-4 production in T cells. In particular, ceramide derivatives with a lauroyl group showed strong inhibitory activities on IL-4 production in both phorbol 12-myristate 13-acetate (PMA)-activated EL4 T cells and antigen-primed primary cells, suggesting that they can be used as compounds for the development of anti-allergic agents.