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Roles of Medicinal Compounds in T Helper Cell-mediated Immunotherapy

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The commitment of T helper (Th) cells to Th1 or Th2 cells is of crucial importance with respective to susceptibility or resistance to particular infections, or to autoimmune diseases 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 significantly regulate the differentiation of Th cells into either Th1 or Th2 subsets. IL-12 and IL-18 promote Th1 differentiation, while IL-4 plays a key role in the differentiation of the precursor $CD4^+$ T cells toward a Th2 phenotype. Recent evidence points to a critical role for IL-12 and IL-18 in the pathogenesis of rodent models of Th1-mediated autoimmune diseases, and for IL-4 and IL-13 in the progression of Th2-mediated allergic diseases. For example, IL-12 was expressed by infiltrating macrophages and synovial lining cells in rheumatoid arthritis (RA) patient, and the median level of circulating IL-12 was higher in patients with RA than in normal healthy people. Exogenous administration of IL-12 exacerbated RA. Therefore, the control of IL-12 and IL-4 production may be a key strategy in modulating specific immune-mediated diseases dominated by type-1 or type 2-cytokine responses.

We have studied the effects of some known medicinal compounds on IL-12 production and also screened plant-originated compounds for regulation of IL-12 production in mouse macrophages. Their action mechanisms were investigated at the molecular levels.

First, we have investigated whether two well-known anti-RA drugs including auranofin and sulfasalazine could regulate Th1/Th2-mediated immune responses. Both auranofin and sulfasalazine significantly inhibited IL-12 production in mouse macrophages *via* inhibition of nuclear factor-κB activation, leading to the shift of Th1 into Th2 responses. In addition, we investigated the effects of curcumin, a natural product of plants obtained from *Curcuma longa* (tumeric), on IL-12 production by mouse splenic macrophages and the subsequent ability of these cells to regulate cytokine production by $CD4^+$ T cells. Pretreatment with curcumin significantly inhibited IL-12 production by macrophages stimulated with either lipopolysaccharide (LPS) or head-killed *Listeria monocytogenes* (HKL). Curcumin-pretreated macrophages reduced their ability to induce IFN-γ and increased the ability to induce IL-4 in Ag-primed $CD4^+$ T cells. Addition of recombinant IL-12 to cultures of curcumin-pretreated

macrophages and $CD4^+$ T cells restored IFN-γ production in $CD4^+$ T cells. The *in vivo* administration of curcumin resulted in the inhibition of IL-12 production by macrophages stimulated *in vitro* with either LPS or HKL, leading to the inhibition of Th1 cytokine profile (decreased IFN-γ and increased IL-4 production) in $CD4^+$ T cells. These findings suggest that curcumin may inhibit Th1 cytokine profile in $CD4^+$ T cells by suppressing IL-12 production in macrophages, and points to a possible therapeutic use of curcumin in the Th1-mediated immune diseases. Other medicinal compounds including parthenolide and hypericin were also know to inhibit IL-12 production in macrophages.

In addition, IL-12 plays a pivotal role in the development of Th1-immune response, which may have therapeutic effects on diseases associated with pathologic Th2 responses such as allergic disorders and asthma. We investigated the effects of berberine, a benzodioxoloquinolizine alkaloid with anti-microbial and anti-tumor activities, on the production of IL-12 p40, an inducible subunit of IL-12, in mouse macrophages. Berberine induced IL-12 p40 production and activation of p38 mitogen-activated protein kinase (MAPK) in dose-dependent manners, which were significantly inhibited by p38 MAPK inhibitors and yohimbine, indicating that p38 MAPK and α_2-adrenergic receptor were involved in the induction of IL-12 p40 production in mouse macrophages by berberine. Furthermore, berberine significantly enhanced IL-12 p40 production in mouse macrophages when combined with lipopolysaccharide, a well-known inducer of IL-12 production. Pretreatment with berberine also induced IL-12 production in both macrophages and dendritic cells, and significantly increased the levels of IL-12 production in lipopolysaccharide (LPS)-stimulated macrophages and in CD40L-stimulated dendritic cells. Importantly, berberine-pretreated macrophages increased their ability to induce IFN-γ and reduced the ability to induce IL-4 in Ag-primed $CD4^+$ T cells. Berberine did not influence the macrophage cell surface expression of the class II MHC molecule, the costimulatory molecules CD80 and CD86, and the adhesion molecule ICAM-1. Addition of a neutralizing anti-IL-12p40 mAb to cultures of berberine-pretreated macrophages and $CD4^+$ T cells restored IL-4 production in Ag-primed $CD4^+$ T cells. The *in vivo* administration of berberine resulted in the enhanced induction of IL-12 production by macrophages when stimulated *in vitro* with LPS or heat-killed *Listeria monocytogenes* (HKL), leading to the inhibition of Th2 cytokine profile (decreased IL-4 and increased IFN-γ production) in Ag-primed $CD4^+$ T cells. These findings may point to a possible therapeutic use of berberine or medicinal plants containing berberine in the Th2 cell-mediated immune diseases such as allergic diseases.

In conclusion, these studies suggest possible therapeutic uses of Th cell-mediated immune response-regulating compounds in diseases dominated by undesired Th1 or Th2 cell-mediated responses.