

# A novel IL-6/IL-12 family cytokine IL-27 and its antitumor activity

## Review Article

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**Abbreviations:** colon 26 (C26); cytotoxic T lymphocyte (CTL); Epstein-Barr virus-induced gene 3 (EBI3); immunoglobulin (Ig); interferon (IFN); interleukin (IL); invariant (i); Janus kinase (JAK); natural killer (NK); receptor (R); signal transducer and activator of transcription (STAT); single-chain (sc) T helper (Th); T-cell cytokine receptor (TCCR)

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## Summary

Interleukin (IL)-12 is a heterodimeric pro-inflammatory cytokine, which plays a critical role in a link between innate and adaptive immunities and in the development of type 1 cell-mediated immunity. Novel heterodimeric cytokines IL-23 and IL-27 with structural and functional similarities to IL-12 have been identified. These novel cytokines establish the IL-6/IL-12 family, whereas they mediate distinct cellular functions and roles, resulting in the coordinate regulation of Th1 development and type 1 cell-mediated immunity. Recent studies have revealed that both IL-23 and IL-27 possess a potent antitumor activity. Although IL-12 has a powerful antitumor activity against various tumors, IL-12 therapy has been limited by its systemic toxicities. Therefore, IL-23 and IL-27 may be alternative attractive candidates as therapeutic agents against tumors. This review summarizes recent advance on the roles of IL-27 in immune regulation and its antitumor activity.

## I. Introduction

Interleukin (IL)-12 is a cytokine that plays a critical role in the interaction between innate and adaptive immunities (Trinchieri, 1995; Gately et al, 1998). IL-12 is produced by antigen-presenting cells such as monocytes/macrophages and dendritic cells (DCs) and acts on T cells and natural killer (NK) cells by inducing proliferation and production of cytokines, especially IFN- $\gamma$ , and by enhancing generation and activity of cytotoxic lymphocytes. IL-12 is a dominant cytokine responsible for the differentiation of T helper (Th)1 cells, which is required for the generation of type 1 cell-mediated immunity. More than 10 years later since IL-12 was identified, two novel cytokines IL-23 and IL-27 with structurally and functionally similarities to IL-12 have been cloned by Dr. Kastelein's group (DNAX Research Institute, Palo Alto, CA) in 2000 and 2002, respectively, by means of computational search in sequence databases with a profile of IL-6 subfamily structure (**Figure 1**)

(Oppmann et al, 2000; Pflanz et al, 2002). Although these novel molecules establish the IL-6/IL-12 family and have various similarities to IL-12, they mediate distinct cellular functions and roles, and coordinately regulate the Th1 development and type 1 cell-mediated immunity (**Figure 2**). IL-23 acts on memory but not naive CD4<sup>+</sup> T cells (Oppmann et al, 2000) and plays a critical role in promoting a distinct T cell activation state characterized by production of IL-17, leading to the development of autoimmune diseases (Aggarwal et al, 2003; Cua et al, 2003; Murphy et al, 2003; Langrish et al, 2005). On the other hand, IL-27 acts on naive but not memory CD4<sup>+</sup> T cells, and plays pivotal roles as a pro-inflammatory cytokine to promote the early initiation of Th1 differentiation and also as an anti-inflammatory cytokine to limit the T cell hyperactivity and production of pro-inflammatory cytokines (Villarino et al, 2004). IL-12 is one of the most powerful antitumor cytokines and the promising data obtained in the pre-clinical models have raised much hope that IL-12 could be a powerful

therapeutic agent against cancer (Brunda et al, 1993; Colombo and Trinchieri, 2002). However, excessive clinical toxicity and modest clinical response in the clinical trials have limited the IL-12 therapy (Marshall, 1995). Recently, we have demonstrated for the first time that IL-27 has a potent antitumor activity (Hisada et al, 2004). In this review, we describe recent advance on the roles of IL-27 in immune regulation and its antitumor activity.

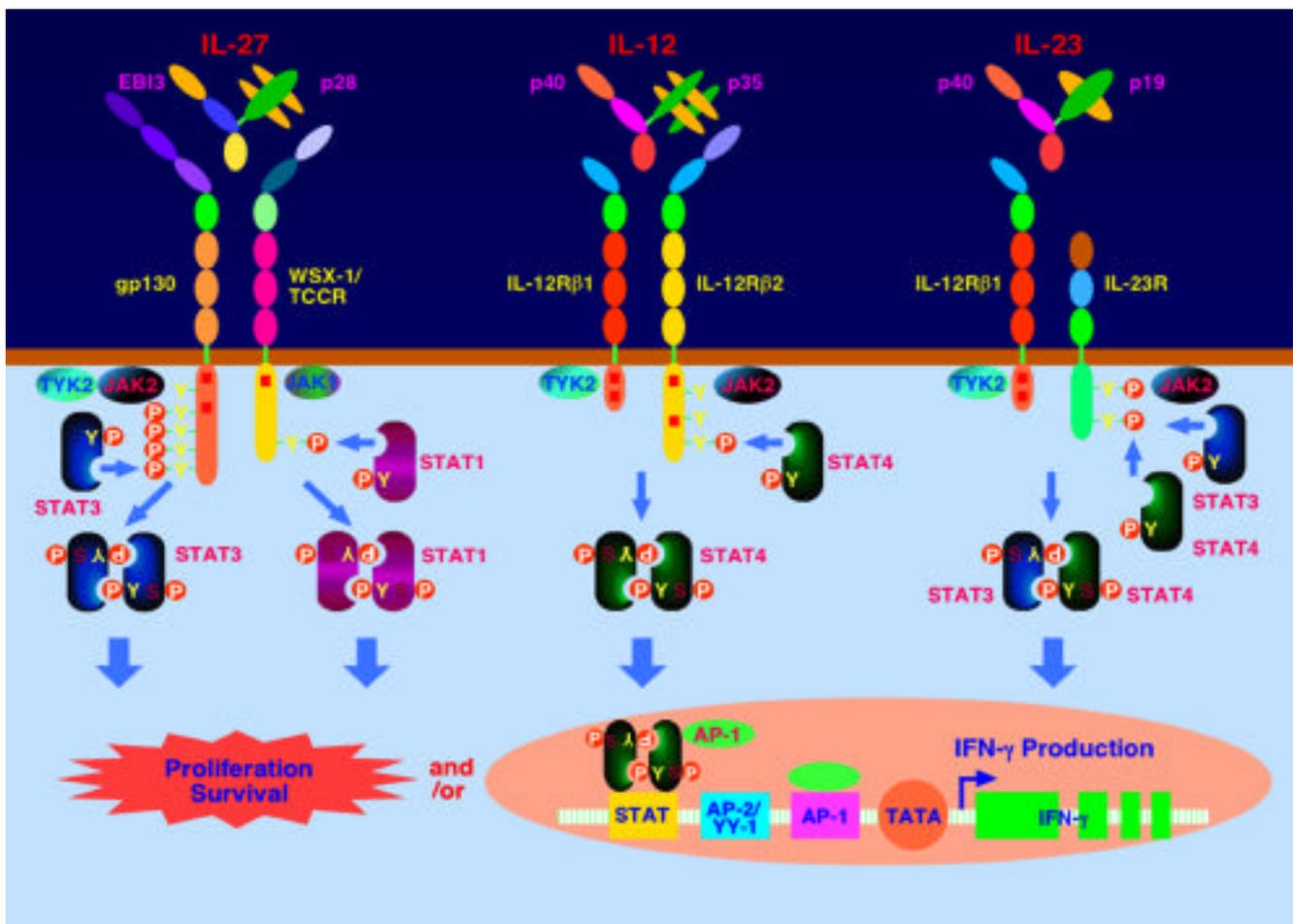
## II. IL-6/IL-12 family cytokines

IL-12 is a heterodimeric cytokine composed of p35 and p40 subunits and plays an immunoregulatory role to promote the type 1 cell-mediated immunity (Trinchieri and Scott, 1995). IL-12 receptor (R) consists of two subunits IL-12R 1 and 2, and IL-12 activates signal transducer and activator of transcription (STAT)4, which binds to

cytoplasmic region of IL-12R 2. The p40 subunit is also covalently bound with a novel p35-related protein p19 to form IL-23 (Oppmann et al, 2000). Receptor for IL-23 is composed of one of IL-12R subunits IL-12R 1 and a novel IL-12R 2-like receptor subunit designated IL-23R (Parham et al, 2002). IL-23 activates STAT3 and STAT4, and IL-23R has a binding domain for STAT4. IL-23 induces strong proliferation of memory T cells but not of naive T cells, whereas IL-12 has no effect on memory T cells (**Table 1**) (Oppmann et al, 2000). Recent studies on the contribution of IL-12 and IL-23 to develop autoimmune diseases demonstrated that IL-23 rather than IL-12 is the essential cytokine (Cua et al, 2003). In addition, IL-23 promotes a distinct T cell activation state characterized by production of IL-17, leading to the development of autoimmune diseases (Aggarwal et al, 2003; Murphy et al, 2003; Langrish et al, 2005).

**Table 1.** Molecular characterization of IL-12, IL-23 and IL-27

	IL-12	IL-23	IL-27
Subunit	p40/p35	p40/p19	EBI3/p28
Receptor	IL-12R 1/IL-12R 2	IL-12R 1/IL-23R	WSX-1(TCCR)/gp130
STAT	STAT4	STAT3/4	STAT1/2/3/4/5
Responsive T cell	Naive	Memory	Naive (Early)

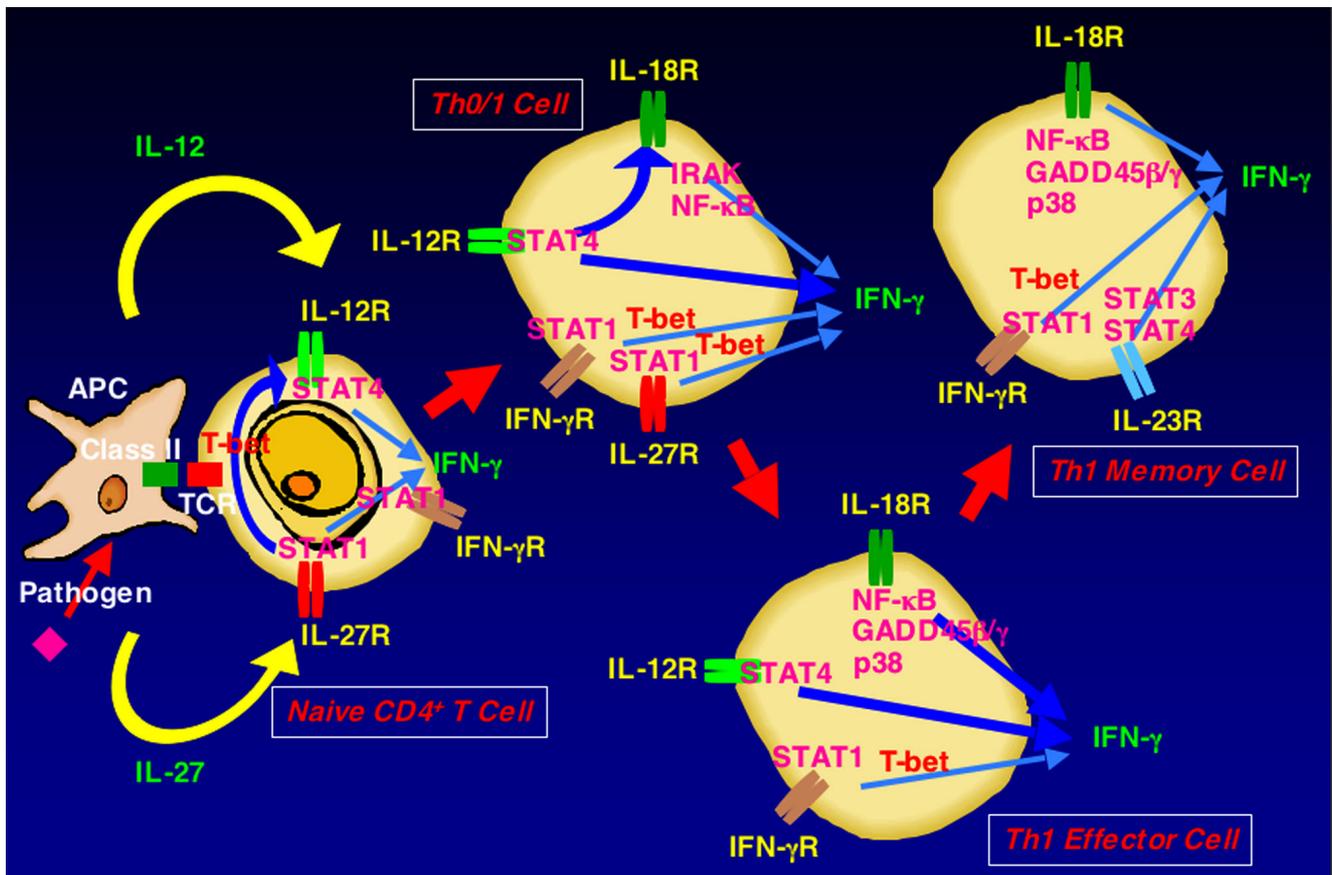


**Figure 1.** The IL-6/IL-12 family cytokines IL-12, IL-23 and IL-27. IL-27 is a unique cytokine that efficiently activates both STAT1 and STAT3, and mediates its biological functions by selectively utilizing these STAT1 and STAT3, which bind to distinct IL-27R subunits, WSX-1 and gp130, respectively.

IL-27 is a heterodimeric cytokine that consists of a newly discovered IL-12 p35-related protein p28 and an IL-12 p40-related protein, Epstein-Barr virus (EBV)-induced gene 3 (EBI3), which had been already identified as one of molecules induced by EBV infection (Pflanz et al, 2002). One of IL-27R subunits is the orphan cytokine receptor WSX-1/T-cell cytokine receptor (TCCR), which has a WSXWS sequence and is homologous to the IL-12R 2 subunit. Recently, gp130, a common receptor subunit for IL-6 family cytokines, has been identified as an another IL-27R subunit and together with WSX-1/TCCR constitutes a functional signal-transducing receptor for IL-27 (Pflanz et al, 2004). IL-27 appears to be produced early by activated antigen-presenting cells and is able to induce clonal proliferation of naive but not memory CD4<sup>+</sup> T cells (Pflanz et al, 2002). IL-27 also induces T-bet and IL-12R 2 expression and synergies with IL-12 in interferon (INF)- production (Figure 2) (Hibbert et al, 2003; Lucas et al, 2003; Takeda et al, 2003; Kamiya et al, 2004). Recent studies on mice lacking one subunit of IL-27R, WSX-1/TCCR, revealed that IL-27 not only promotes the early initiation of Th1 responses (Chen et al, 2000; Yoshida et al, 2001), but also limits the T cell hyperactivity and production of pro-inflammatory cytokines (Hamano et al, 2003; Villarino et al, 2003).

### III. IL-27 signaling

IL-27 is a unique cytokine that efficiently activates both STAT1 and STAT3, and mediates its biological functions by selectively utilizing these STAT1 and STAT3, which bind to distinct IL-27R subunits, WSX-1 and gp130, respectively (Figure 1). IL-27 activates Janus kinase (JAK)1, -2, TYK2, STAT1, -2, -3, -4, -5 in naive CD4<sup>+</sup> T cells (Table 1) (Hibbert et al, 2003; Takeda et al, 2003; Villarino et al, 2003; Kamiya et al, 2004). Tyrosine phosphorylation of STAT1 by IL-27 is mediated through a cytoplasmic region of WSX-1 (Takeda et al, 2003) and required for T-bet and IL-12R 2 expression, resulting in synergistic IFN- production with IL-12 (Kamiya et al, 2004). On the other hand, tyrosine phosphorylation of STAT3 is considered to be mediated through a cytoplasmic region of gp130 and presumably necessary for IL-27-induced proliferation (Kamiya et al, 2004). Thus, IL-27 possesses at least two signal transduction pathways; one is to lead to T-bet expression through WSX-1 and STAT1 activation and the other is to lead to proliferation through gp130 and STAT3 activation. The JAK/STAT signaling pathway is most important for mediating biological responses induced by many cytokines (Ihle, 1996; Darnell, 1997; Leonard and O'Shea, 1998). Selective usage of members of the JAK and STAT families by a given cytokine receptor is considered to be responsible for the specificity of cytokine action. However, if two cytokines activate the same JAK/STAT



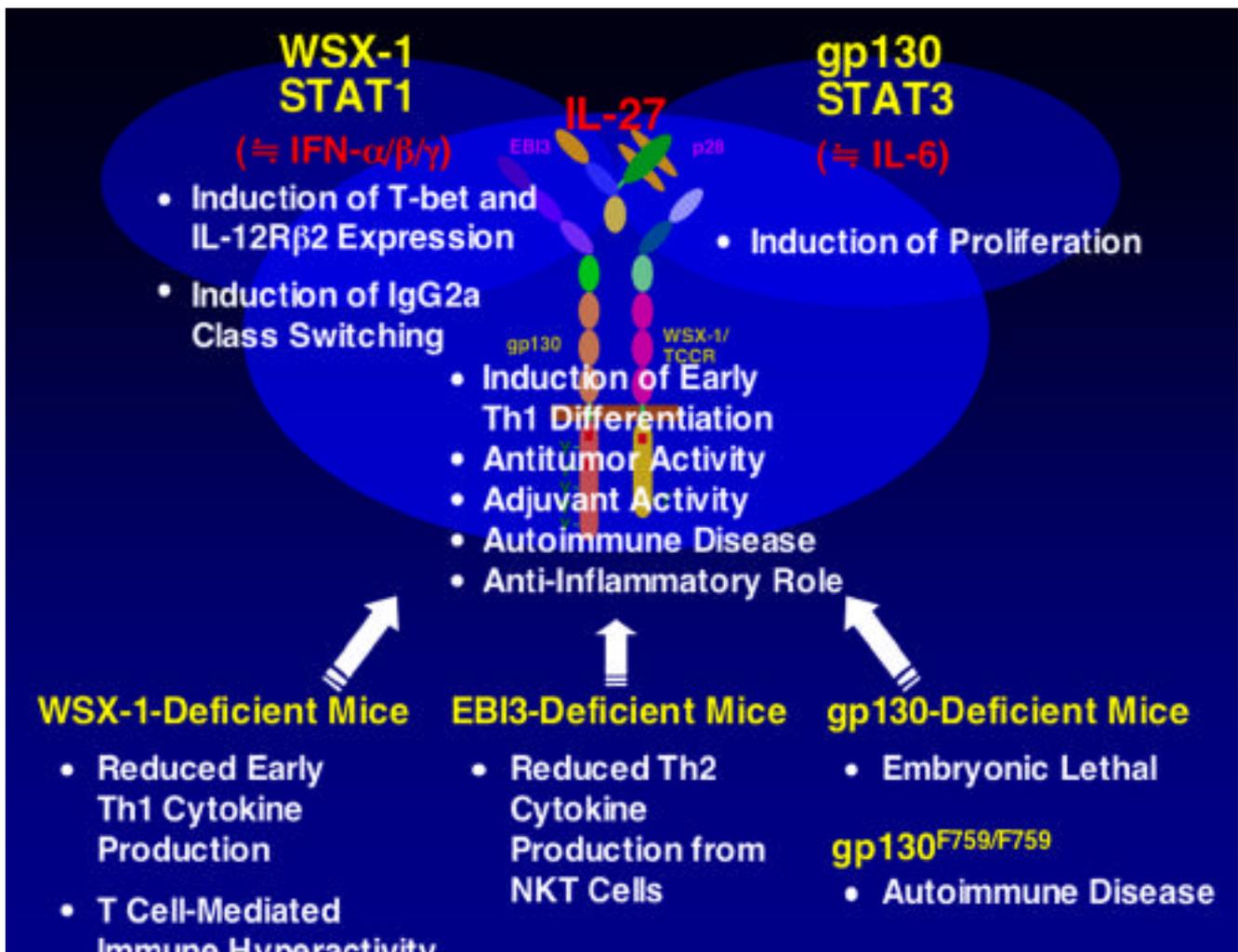
**Figure 2.** Coordinated regulation of Th1 differentiation by the IL-6/IL-12 family cytokines IL-12, IL-23 and IL-27. IL-27 acts on naive CD4<sup>+</sup> T cells prior to IL-12, IL-12 acts on activated CD4<sup>+</sup> T cells and IL-23 acts on memory CD4<sup>+</sup> T cells, resulting in the coordinated regulation of development of type 1 cell-mediated immunity.

signaling molecules, it is possible to expect similar biological actions between them. IFN- $\gamma$  utilizes JAK1, TYK2, STAT1, and -2, and IFN- $\alpha/\beta$  utilizes JAK1, -2 and STAT1 (Fu et al, 1992; Schindler et al, 1992; Shuai et al, 1992; Velazquez et al, 1992; Muller et al, 1993; Watling et al, 1993; Leonard and O'Shea, 1998). IL-6 activates JAK1, -2, TYK2, STAT1 and in particular STAT3 (Lutticken et al, 1994; Stahl et al, 1994; Heinrich et al, 1998). Notably, these patterns are similar to those activated by IL-27 (Table 1). Therefore, IL-27 may have similar biological actions to IFN- $\gamma$  and IL-6 in STAT1- and STAT3-dependent manners, respectively. Indeed, IL-27 augments T-bet expression in T cells (Takeda et al, 2003) and induces immunoglobulin (Ig)G2a class switching in B cells (Yoshimoto et al, 2004) as does IFN- $\gamma$ .

#### IV. Roles of IL-27 in immune regulation

The role of IL-27 in regulating immune response is complex with its stimulatory and inhibitory effects (Figure 3) (Villarino et al, 2004). IL-27 is considered to

play a role in the early regulation of Th1 initiation, whereas the exact role of IL-27 in Th1 differentiation and its molecular mechanism remain unclear. Previous studies on in vitro Th1/Th2 differentiation analyses using TCCR (Chen et al, 2000)/WSX-1 (Yoshida et al, 2001)-deficient CD4<sup>+</sup> T cells and kinetic analyses on the induction of EB13 and p28 mRNA expression in monocytes/macrophages and DCs (Pflanz et al, 2002) suggest that IL-27 plays an important role in the early initiation of Th1 responses by inducing T-bet and subsequent IL-12R $\beta$ 2 expression but inhibiting GATA-3 expression prior to the action of IL-12 emerges (Lucas et al, 2003; Takeda et al, 2003; Kamiya et al, 2004). Moreover, susceptibilities of WSX-1/TCCR-deficient mice to various pathogens have been investigated. In agreement with the in vitro studies indicating a role for IL-27 in promoting the initiation of Th1 differentiation, WSX-1/TCCR-deficient mice have enhanced susceptibility to infection with intracellular pathogens.



**Figure 3.** Roles of IL-27 in immune regulation. IL-27 plays pivotal roles as a pro-inflammatory cytokine to promote the early initiation of Th1 differentiation and also as an anti-inflammatory cytokine to limit the T cell hyperactivity and production of pro-inflammatory cytokines. IL-27 may have similar biological actions to IFN- $\gamma$  and IL-6 in STAT1- and STAT3-dependent manners, respectively.

Early in infection with a protozoan parasite *Leishmania major*, WSX-1-deficient mice are remarkably susceptible to the infection showing impaired IFN- production and advanced lesion development (Yoshida et al, 2001; Artis et al, 2004). Similarly, reduced production of IFN- is observed when WSX-1-deficient mice are challenged with an avirulent strain of mycobacterium (BCG) (Yoshida et al, 2001). TCCR-deficient mice also have increased susceptibility to infection with *Listeria monocytogenes* showing markedly reduced antigen-specific IgG2a production, which is dependent on Th1 cells (Chen et al, 2000). However, WSX-1/TCCR is not essential to develop the protective Th1 responses. After *Leishmania major* or BCG infection, defects in pathogen-induced IFN- production are transient, and as each disease progresses WSX-1-deficient mice generate Th1 type responses and control infection like wild-type mice (Yoshida et al, 2001; Artis et al, 2004). Moreover, recent studies revealed that WSX-1 can also deliver inhibitory signals to limit the magnitude and duration of type I-mediated inflammatory responses. WSX-1-deficient mice infected with *Toxoplasma gondii* parasites generate a robust Th1 response as wild-type mice, but fail to regulate the intensity of effector T cells responses, resulting in hyperactive CD4<sup>+</sup> T cells and a lethal inflammatory cytokine production (Villarino et al, 2003). *Toxoplasma gondii* promotes strong innate immune responses that lead to systemic IL-12 levels early during infection, whereas acute *Leishmania major* induces much less IL-12 production (Scott and Hunter, 2002). Therefore, key differences between infection with *Leishmania major* or BCG and that with *Toxoplasma gondii* might be the induction level of IL-12 and/or the responsible cell population; CD4<sup>+</sup> T cells vs macrophages.

Studies on mice lacking of other IL-27/IL-27R subunits, EBI3 and gp130, were previously reported. Deficient mice of one of IL-27 subunits EBI3 exhibit a reduced number of invariant (i)NKT cells and produce much lower levels of IL-4 than wild-type mice, whereas the production of IFN- is only moderately and transiently lower than in wild-type mice (Nieuwenhuis et al, 2002). EBI3-deficient mice are resistant to oxazolone-induced colitis, a model mediated by Th2 cytokine production initiated by iNKT cells, whereas they are as susceptible as wild-type mice to a Th1-mediated colitis model induced by trinitrobenzene sulfonic acid. These data suggest that an EBI3-dependent factor possibly different from IL-27 might be involved in IL-4-mediated Th2 responses. Deficient mice of the other IL-27 subunit gp130 show embryonic lethality. However, it was shown that a mouse line in which the negatively regulated binding site for Src homology 2 domain tyrosine phosphatase (SHP-2) and suppressor of cytokine signaling (SOCS)3 in gp130, tyrosine 759, is mutated to phenylalanine, gp130<sup>F759P</sup>, spontaneously develop a rheumatoid arthritis (RA)-like joint disease (Atsumi et al, 2002).

Several studies which support a role of IL-27 in the augmentation of type 1 cell-mediated immunity have been reported. IL-12 is well known to possess a strong adjuvant activity, Matsui et al, (2004) have evaluated the adjuvant activity of IL-23 and IL-27 in the prime-boost

immunization consisting of priming and the first boosting with the hepatitis C virus (HCV) core expression plasmid, followed by a second boosting with recombinant adenovirus expressing HCV core. Coadministration of either an IL-23 or IL-27 expression plasmid in the prime-boost immunization enhances induction of HCV-specific cytotoxic T lymphocytes (CTLs) and leads to dramatic increases in the numbers of IFN- producing HCV-specific CD8<sup>+</sup> T cells, indicating that IL-23 and IL-27 have a potent adjuvant activity for the induction of HCV-specific CTLs. Yoshimoto et al, (2004) have evaluated the role of IL-27 in B cells and demonstrated that primary spleen B cells express functional IL-27R and that the stimulation of these B cells by IL-27 induces T-bet expression and IgG2a, but not IgG1, class switching in STAT1-dependent but IFN- independent manner. In contrast, IL-27 inhibits IgG1 class switching induced by IL-4 in activated B cells. The IL-27-induced IgG2a class switching is highly dependent on T-bet in response to T-independent stimuli such as LPS. Thus, IL-27 may be a novel candidate as a therapeutic agent against diseases such as allergic disorders by not only regulating Th1 differentiation but also directly acting on B cells and inducing IgG2a class switching. Moreover, it has been very recently demonstrated that neutralizing the in vivo function of IL-27 by antibody against p28 suppresses not only development of autoimmune diseases such as adjuvant-induced arthritis and experimental autoimmune encephalomyelitis (EAE) but also their ongoing diseases (Goldberg et al, 2004a, b). These results suggest that IL-27 affects not only naive T cells undergoing antigen-specific activation, but also effector/memory Th1 cells, although IL-27 has been considered to be involved only in the initiation of Th1 differentiation. Further studies are necessary to explain the difference.

## V. Antitumor activity of IL-23

Since IL-23 has structural and functional similarities to IL-12, which is one of the most powerful antitumor cytokines in vivo, it was expected that IL-23 might be an attractive candidate as an antitumor agent. Wang et al, (2003) examined whether murine colon carcinoma cells colon 26 (C26) that are retrovirally transduced with the p19-linked p40 gene (C26-IL-23) can produce antitumor effects in inoculated mice. The growth of C26-IL-23 tumors developed in immunocompetent mice is significantly retarded and the tumors disappear thereafter. Spleen cells from the mice that received C26-IL-23 cells produce significant amounts of IFN- , when they are cultured with irradiated C26 tumors but not irrelevant cells. Depletion of CD8<sup>+</sup> T cells suppresses the production of IFN- . The mice that have rejected C26-IL-23 tumors are resistant to subsequent challenge of parent but not irrelevant tumor cells. C26-IL-23 tumors are not rejected in nude mice but the growth is retarded compared to parent tumors. Treatment of nude mice with anti-asialoGM1 antibody dose not influence the growth of C26-IL-23 tumors.

Lo et al, (2003) also reported on the antitumor activity of IL-23 using murine CT26 colon

adenocarcinoma and B16F1 melanoma cells, which are engineered using retroviral vectors to release scIL-23. scIL-23-transduced CT26 cells grow progressively in immunocompetent mice until day 26, then the tumors start to regress in most mice, resulting in a final 70% rate of complete tumor rejection. scIL-23 transduction also significantly suppresses lung metastases of CT26 and B16F1 tumor cells. In addition, mice that rejected scIL-23-transduced tumors develop a memory response against subsequent wild-type tumor challenge. Compared with scIL-12-expressing CT26 cells, scIL-23-transduced tumors lack the early response, but achieve comparable antitumor and antimetastatic activity. Tumor challenge studies in immunocompromised hosts and in mice selectively depleted of various lymphocyte populations revealed that CD8<sup>+</sup> T cells, but not CD4<sup>+</sup> T cells or NK cells, are crucial for the antitumor activity of IL-23.

Taken together, these studies suggest that expression of IL-23 in tumors induces T cell-dependent antitumor effects and systemic protective immunity like that of IL-12.

## VI. Antitumor activity of IL-27

### A. Murine colon carcinoma 26

Since IL-27 has several similarities to IL-12 and plays a role in the initiation of Th1 differentiation, Hisada et al, (2004) have evaluated the antitumor activity of IL-27 against a murine tumor model of colon carcinoma colon 26 (C26) and revealed that IL-27 possesses a potent antitumor activity. C26 tumor cells, which are transduced with the single-chain (sc)IL-27 cDNA and become secreting IL-27 (C26-IL-27), exhibit a minimal tumor growth in vivo, and all mice inoculated with these tumor cells survive with a complete tumor remission. Inoculation of mice with C26-IL-27 tumors induces enhanced IFN- $\gamma$  production and CTL activity against C26 tumors in spleen cells. Recovered mice from the inoculation show a tumor-specific protective immunity to the following challenge with parental C26 tumors. The antitumor activity of IL-27 is almost diminished in nude mice, and depletion of CD8<sup>+</sup> T cells and neutralization of IFN- $\gamma$  in immunocompetent mice reduce the antitumor activity. Moreover, the antitumor activity is abolished in T-bet-deficient mice, while unexpectedly it is still observed in STAT4-deficient mice. These results suggest that IL-27 has potent abilities to induce tumor-specific antitumor activity and protective immunity, and that the antitumor activity is mediated through mainly CD8<sup>+</sup> T cells, IFN- $\gamma$ , and T-bet, but not STAT4.

IL-27 acts on naive CD4<sup>+</sup> T cells and regulates only the initiation phase of Th1 responses but not the induction and maintenance phases of effector Th1 responses (Chen et al, 2000; Yoshida et al, 2001; Pflanz et al, 2002; Takeda et al, 2003). This is a contrast to IL-12 that is involved in the induction and maintenance phases of effector Th1 responses, therefore presumably sometimes leading to the excessive toxicity in vivo (Car et al, 1995; Marshall, 1995; Ryffel, 1997; Car et al, 1999). Thus, it might be possible to expect the lower toxicity in the treatment with IL-27. Indeed, during the IL-27 treatment, any apparent adverse

effects such as splenomegaly and liver injury with elevated serum glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) activities and intensive mononuclear cell infiltration into liver were not observed (unpublished observation), which are seen with IL-12 treatment (Car et al, 1995; Ryffel, 1997; Car et al, 1999). Further studies with systemic administration of IL-27 and detailed analyses in other organs are necessary to determine the absence or presence of adverse effects in the IL-27 treatment.

### B. Murine neuroblastoma TBJ

Recently, Salcedo et al, (2004) have also reported that IL-27 exerts a potent antitumor activity in vivo using TBJ neuroblastoma cells, which are engineered to overexpress scIL-27 (TBJ-IL-27). TBJ-IL-27 tumors show markedly delayed growth compared with control mice, and complete durable tumor regression is observed in >90% of mice bearing either s.c. or orthotopic intra-adrenal tumors, and in 40% of mice bearing induced metastatic disease. The majority of mice cured of TBJ-IL-27 tumors are resistant to tumor rechallenge. Furthermore, TBJ-IL-27 tumors are heavily infiltrated by CD8<sup>+</sup> T cells. Draining lymph node-derived lymphocytes from mice bearing s.c. TBJ-IL-27 tumors are primed to proliferate more readily when cultured ex vivo with anti-CD3/anti-CD28 compared with lymphocytes from mice bearing control tumors, and to secrete higher levels of IFN- $\gamma$ . In addition, marked enhancement of local IFN- $\gamma$  gene expression and potent up-regulation of cell surface MHC class I expression are noted within TBJ-IL-27 tumors compared with control tumors. Functionally, these alterations occur in conjunction with the generation of tumor-specific CTL reactivity in mice bearing TBJ-IL-27 tumors, and the induction of tumor regression via mechanisms that are critically dependent on CD8<sup>+</sup>, but not CD4<sup>+</sup> T cells or NK cells.

Collectively, these two studies clearly indicate that IL-27 could be applied therapeutically to potentiate the host antitumor immune response in patients with malignancy.

## VII. Conclusion

Effective eradication of established tumors and generation of a long-lasting systemic immune response with a simple delivery system are important goals for cancer immunotherapy. Cytokines are the most widely and extensively studied immunostimulatory agents in the cancer therapy (Leroy et al, 1998). For clinical application, local and systemic administration of IL-12 protein has been studied in various murine models (Rakhmilevich et al, 1996; Tahara et al, 1996; Leroy et al, 1998; Watanabe et al, 1999) and in Phase I/II human trials (Golab and Zagodzoon, 1999; Rook et al, 1999). However, IL-12 therapy has been limited by systemic toxicities such as hepatomegaly, splenomegaly, leucopenia, myelodepression, lung edema, and gastrointestinal toxicity (Car et al, 1995; Marshall, 1995; Ryffel, 1997; Car et al, 1999). Therefore, application of other novel cytokines with potent antitumor activity but devoid of systemic

toxicity is an alternative immunotherapeutic approach. Thus, recently identified novel members of the IL-6/IL-12 family, IL-23 and IL-27, which play pivotal roles in the coordinated regulation of Th1 differentiation and type 1 cell-mediated immunity, could be attractive candidates as an agent applicable to the cancer therapy.

## References

- Aggarwal S, Ghilardi N, Xie MH, de Sauvage FJ, and Gurney AL (2003) Interleukin-23 promotes a distinct CD4 T cell activation state characterized by the production of interleukin-17. **J Biol Chem** 278, 1910-4.
- Artis D, Johnson LM, Joyce K, Saris C, Villarino A, Hunter CA, and Scott P (2004) Cutting Edge: Early IL-4 production governs the requirement for IL-27-WSX-1 signaling in the development of protective Th1 cytokine responses following *Leishmania major* infection. **J Immunol** 172, 4672-5.
- Atsumi T, Ishihara K, Kamimura D, Ikushima H, Ohtani T, Hirota S, Kobayashi H, Park SJ, Saeki Y, Kitamura Y, and Hirano T (2002) A point mutation of Tyr-759 in interleukin 6 family cytokine receptor subunit gp130 causes autoimmune arthritis. **J Exp Med** 196, 979-90.
- Brunda MJ, Luistro L, Warriar RR, Wright RB, Hubbard BR, Murphy M, Wolf SF, and Gately MK (1993) Antitumor and antimetastatic activity of interleukin 12 against murine tumors. **J Exp Med** 178, 1223-30.
- Car BD, Eng VM, Lipman JM, and Anderson TD (1999) The toxicology of interleukin-12: a review. **Toxicol Pathol** 27, 58-63.
- Car BD, Eng VM, Schnyder B, LeHir M, Shakhov AN, Woerly G, Huang S, Aguet M, Anderson TD, and Ryffel B (1995) Role of interferon-gamma in interleukin 12-induced pathology in mice. **Am J Pathol** 147, 1693-707.
- Chen Q, Ghilardi N, Wang H, Baker T, Xie MH, Gurney A, Grewal IS, and de Sauvage FJ (2000) Development of Th1-type immune responses requires the type I cytokine receptor TCCR. **Nature** 407, 916-20.
- Colombo MP, and Trinchieri G (2002) Interleukin-12 in anti-tumor immunity and immunotherapy. **Cytokine Growth Factor Rev** 13, 155-68.
- Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, et al (2003) Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. **Nature** 421, 744-8.
- Darnell JE, Jr. (1997) STATs and gene regulation. **Science** 277, 1630-5.
- Fu XY, Schindler C, Improtta T, Aebersold R, and Darnell JE, Jr. (1992) The proteins of ISGF-3, the interferon alpha-induced transcriptional activator, define a gene family involved in signal transduction. **Proc Natl Acad Sci U S A** 89, 7840-3.
- Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, and Presky DH (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. **Annu Rev Immunol** 16, 495-521.
- Golab J, and Zagozdzon R (1999) Antitumor effects of interleukin-12 in pre-clinical and early clinical studies (Review). **Int J Mol Med** 3, 537-44.
- Goldberg R, Wildbaum G, Zohar Y, Maor G, and Karin N (2004a) Suppression of ongoing adjuvant-induced arthritis by neutralizing the function of the p28 subunit of IL-27. **J Immunol** 173, 1171-8.
- Goldberg R, Zohar Y, Wildbaum G, Geron Y, Maor G, and Karin N (2004b) Suppression of ongoing experimental autoimmune encephalomyelitis by neutralizing the function of the p28 subunit of IL-27. **J Immunol** 173, 6465-71.
- Hamano S, Himeno K, Miyazaki Y, Ishii K, Yamanaka A, Takeda A, Zhang M, Hisaeda H, Mak TW, Yoshimura A, and Yoshida H (2003) WSX-1 is required for resistance to *Trypanosoma cruzi* infection by regulation of proinflammatory cytokine production. **Immunity** 19, 657-67.
- Heinrich PC, Behrmann I, Muller-Newen G, Schaper F, and Graeve L (1998) Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. **Biochem J** 334 ( Pt 2), 297-314.
- Hibbert L, Pflanz S, De Waal Malefyt R, and Kastelein RA (2003) IL-27 and IFN- signal via Stat1 and Stat3 and induce T-Bet and IL-12Rbeta2 in naive T cells. **J Interferon Cytokine Res** 23, 513-22.
- Hisada M, Kamiya S, Fujita K, Belladonna ML, Aoki T, Koyanagi Y, Mizuguchi J, and Yoshimoto T (2004) Potent antitumor activity of interleukin-27. **Cancer Res** 64, 1152-6.
- Ihle JN (1996) STATs: signal transducers and activators of transcription. **Cell** 84, 331-4.
- Kamiya S, Owaki T, Morishima N, Fukai F, Mizuguchi J, and Yoshimoto T (2004) An indispensable role for STAT1 in IL-27-induced T-bet expression but not proliferation of naive CD4<sup>+</sup> T cells. **J Immunol** 173, 3871-7.
- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, and Cua DJ (2005) IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. **J Exp Med** 201, 233-40.
- Leonard WJ, and O'Shea JJ (1998) Jaks and STATs: biological implications. **Annu Rev Immunol** 16, 293-322.
- Leroy P, Slos P, Homann H, Erbs P, Poitevin Y, Regulier E, Colonna FQ, Devauchelle P, Roth C, Pavirani A, and Mehtali M (1998) Cancer immunotherapy by direct in vivo transfer of immunomodulatory genes. **Res Immunol** 149, 681-4.
- Lo CH, Lee SC, Wu PY, Pan WY, Su J, Cheng CW, Roffler SR, Chiang BL, Lee CN, Wu CW, and Tao MH (2003) Antitumor and antimetastatic activity of IL-23. **J Immunol** 171, 600-7.
- Lucas S, Ghilardi N, Li J, and de Sauvage FJ (2003) IL-27 regulates IL-12 responsiveness of naive CD4<sup>+</sup> T cells through Stat1-dependent and -independent mechanisms. **Proc Natl Acad Sci U S A** 100, 15047-52.
- Lutticken C, Wegenka UM, Yuan J, Buschmann J, Schindler C, Ziemiecki A, Harpur AG, Wilks AF, Yasukawa K, Taga T, and et al (1994) Association of transcription factor APRF and protein kinase Jak1 with the interleukin-6 signal transducer gp130. **Science** 263, 89-92.
- Marshall E (1995) Cancer trial of interleukin-12 halted. **Science (Wash DC)** 268, 1555.
- Matsui M, Moriya O, Belladonna ML, Kamiya S, Lemonnier FA, Yoshimoto T, and Akatsuka T (2004) Adjuvant activities of novel cytokines, interleukin-23 (IL-23) and IL-27, for induction of hepatitis C virus-specific cytotoxic T lymphocytes in HLA-A\*0201 transgenic mice. **J Virol** 78, 9093-104.
- Muller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, Harpur AG, Barbier G, Witthuhn BA, Schindler C, et al (1993) The protein tyrosine kinase JAK1 complements a mutant cell line defective in the interferon-alpha/beta and gamma signal transduction pathways. **Nature** 366, 129-35.
- Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, and Cua DJ (2003) Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. **J Exp Med** 198, 1951-7.

- Nieuwenhuis EE, Neurath MF, Corazza N, Iijima H, Trgovcich J, Wirtz S, Glickman J, Bailey D, Yoshida M, Galle PR, et al (2002) Disruption of T helper 2-immune responses in Epstein-Barr virus-induced gene 3-deficient mice. **Proc Natl Acad Sci U S A** 99, 16951-6.
- Oppmann B, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, et al (2000) Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. **Immunity** 13, 715-25.
- Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, et al (2002) A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. **J Immunol** 168, 5699-708.
- Pflanz S, Hibbert L, Mattson J, Rosales R, Vaisberg E, Bazan JF, Phillips JH, McClanahan TK, de Waal Malefyt R, and Kastelein RA (2004) WSX-1 and glycoprotein 130 constitute a signal-transducing receptor for IL-27. **J Immunol** 172, 2225-31.
- Pflanz S, Timans JC, Cheung J, Rosales R, Kanzler H, Gilbert J, Hibbert L, Churakova T, Travis M, Vaisberg E, et al (2002) IL-27, a heterodimeric cytokine composed of EB13 and p28 protein, induces proliferation of naive CD4(+) T cells. **Immunity** 16, 779-90.
- Rakhmilevich AL, Turner J, Ford MJ, McCabe D, Sun WH, Sondel PM, Grota K, and Yang NS (1996) Gene gun-mediated skin transfection with interleukin 12 gene results in regression of established primary and metastatic murine tumors. **Proc Natl Acad Sci U S A** 93, 6291-6.
- Rook AH, Wood GS, Yoo EK, Elenitsas R, Kao DM, Sherman ML, Witmer WK, Rockwell KA, Shane RB, Lessin SR, and Vonderheid EC (1999) Interleukin-12 therapy of cutaneous T-cell lymphoma induces lesion regression and cytotoxic T-cell responses. **Blood** 94, 902-8.
- Ryffel B (1997) Interleukin-12: role of interferon-gamma in IL-12 adverse effects. **Clin Immunol Immunopathol** 83, 18-20.
- Salcedo R, Stauffer JK, Lincoln E, Back TC, Hixon JA, Hahn C, Shafer-Weaver K, Malyguine A, Kastelein R, and Wigginton JM (2004) IL-27 mediates complete regression of orthotopic primary and metastatic murine neuroblastoma tumors: role for CD8+ T cells. **J Immunol** 173, 7170-82.
- Schindler C, Fu XY, Improta T, Aebersold R, and Darnell JE, Jr. (1992) Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins that are activated by interferon alpha. **Proc Natl Acad Sci U S A** 89, 7836-9.
- Scott P, and Hunter CA (2002) Dendritic cells and immunity to leishmaniasis and toxoplasmosis. **Curr Opin Immunol** 14, 466-70.
- Shuai K, Schindler C, Prezioso VR, and Darnell JE, Jr. (1992) Activation of transcription by IFN- $\gamma$ : tyrosine phosphorylation of a 91-kD DNA binding protein. **Science** 258, 1808-12.
- Stahl N, Boulton TG, Farruggella T, Ip NY, Davis S, Witthuhn BA, Quelle FW, Silvennoinen O, Barbieri G, Pellegrini S, and et al (1994) Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. **Science** 263, 92-5.
- Tahara H, Zitvogel L, Storkus WJ, Robbins PD, and Lotze MT (1996) Murine models of cancer cytokine gene therapy using interleukin-12. **Ann N Y Acad Sci** 795, 275-83.
- Takeda A, Hamano S, Yamanaka A, Hanada T, Ishibashi T, Mak TW, Yoshimura A, and Yoshida H (2003) Cutting edge: Role of IL-27/WSX-1 signaling for induction of T-bet through activation of STAT1 during initial Th1 commitment. **J Immunol** 170, 4886-90.
- Trinchieri G (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions that bridge innate resistance and antigen-specific adaptive immunity. **Annu Rev Immunol** 13, 251-76.
- Trinchieri G, and Scott P (1995) Interleukin-12: a proinflammatory cytokine with immunoregulatory functions. **Res Immunol** 146, 423-31.
- Velazquez L, Fellous M, Stark GR, and Pellegrini S (1992) A protein tyrosine kinase in the interferon alpha/beta signaling pathway. **Cell** 70, 313-22.
- Villarino A, Hibbert L, Lieberman L, Wilson E, Mak T, Yoshida H, Kastelein RA, Saris C, and Hunter CA (2003) The IL-27R (WSX-1) is required to suppress T cell hyperactivity during infection. **Immunity** 19, 645-55.
- Villarino AV, Huang E, and Hunter CA (2004) Understanding the pro- and anti-inflammatory properties of IL-27. **J Immunol** 173, 715-20.
- Wang YQ, Ugai S, Shimozaoto O, Yu L, Kawamura K, Yamamoto H, Yamaguchi T, Saisho H, and Tagawa M (2003) Induction of systemic immunity by expression of interleukin-23 in murine colon carcinoma cells. **Int J Cancer** 105, 820-4.
- Watanabe M, Fenton RG, Wigginton JM, McCormick KL, Volker KM, Fogler WE, Roessler PG, and Wiltrout RH (1999) Intradermal delivery of IL-12 naked DNA induces systemic NK cell activation and Th1 response in vivo that is independent of endogenous IL-12 production. **J Immunol** 163, 1943-50.
- Watling D, Guschin D, Muller M, Silvennoinen O, Witthuhn BA, Quelle FW, Rogers NC, Schindler C, Stark GR, Ihle JN, and et al (1993) Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. **Nature** 366, 166-70.
- Yoshida H, Hamano S, Senaldi G, Covey T, Faggioni R, Mu S, Xia M, Wakeham AC, Nishina H, Potter J, et al (2001) WSX-1 is required for the initiation of Th1 responses and resistance to L. major infection. **Immunity** 15, 569-78.
- Yoshimoto T, Okada K, Morishima N, Kamiya S, Owaki T, Asakawa M, Iwakura Y, Fukai F, and Mizuguchi J (2004) Induction of IgG2a class switching in B cells by IL-27. **J Immunol** 173, 2479-85.



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