Review

Paradoxical Roles of IL-4 in Tumor Immunity

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Interleukin (IL)-4 is a crucial cytokine in tumor immunology. In the initial murine experiments, IL-4 exhibited potent anti-tumor ability. Tumors genetically modified to produce IL-4 were rejected, while parental tumors grew progressively. Mice rejected IL-4-producing tumors got long-lasting anti-tumor immunity. The comparative study showed that IL-4 induced the most effective immune response among several cytokines in both prophylactic and therapeutic models. All of these indicate IL-4 has strong potential as a tumor therapy agent. However, contrary evidence indeed exists, and is becoming more and more abundant which shows IL-4 is a tumor-promoting molecule. IL-4 amounts are usually elevated in human cancer patients. IL-4 knockout mice are more resistant to tumor challenge than IL-4 competent mice. Furthermore, tumor cells of various histological origins often express increased levels of IL-4 receptor in comparison to their normal counterparts. By carefully examining presently available data, we found the effects of IL-4 in tumor immunity are closely related to its sources, expressing time and dose, as well as the molecular and cellular environments. In this mini-review, we concentrate on illustrating the paradoxical roles and underlying mechanisms of IL-4 in tumor immunity and try to understand how one molecule has opposite effects. *Cellular & Molecular Immunology*. 2009;6(6):415-422.

Key Words: endogenous IL-4, exogenous IL-4, tumor immunity

The paradox of IL-4 in tumor immunity

Three years after the cloning of IL-4 gene (1, 2), Tepper et al. expressed this molecule in two tumor cell lines, plasmacytoma J558L and mammary adenocarcinoma K485 (3). Exciting things happened. All J558L tumors producing high amounts of IL-4 and 80% of J558L tumors producing low amounts of IL-4 were rejected, while the parental tumors, from which IL-4-secreting tumor cells were derived, and the mock tumors, which also carried the genetically introduced vector but did not produce IL-4, grew rapidly. Tumors that occasionally escaped the influence of IL-4 were found to lose IL-4 genes. Simultaneously, the growth of IL-4-producing K485 tumors was also inhibited, even in the T cell-deficient nu/nu mice (3). Mice rejected IL-4-secreting tumors got a long-lasting anti-tumor immunity and rejected subsequent challenge of parental tumor cells (4). Interestingly, inoculation of IL-4-secreting tumors led to the rejection of non-related tumors without IL-4 producing ability in mixed tumor transplantation experiment (3). In the following years, IL-4, as a potent anti-tumor agent, was demonstrated in different tumor models, including renal cancer (4), colorectal cancer (4, 5), spontaneous adenocarcinoma (6), colon carcinoma (7, 8), fibrosarcoma (9, 10) and melanoma (9). Okada et al. compared the anti-tumor abilities of several cytokines using rat glioma (11). They transferred the cDNA of granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon alpha (IFNα), IL-12 and IL-4 into rat 9 L tumor cells. Then, they immunized rats by intradermal injection of 2×10^6 9L cells producing the above cytokines and 28 days later, challenged these rats with 1×10^5 parental 9 L tumor cells. Ninty percent rats vaccinated with 9L-IL-4 survived a subsequent challenge of parent tumor cells, much more than rats vaccinated with cells producing other cytokines (40% or 0%). Moreover, IL-4 was also the most effective among the cytokines in a therapeutic model as 43% of rats bearing 3-day 9L tumors survived >100 days after 9L-IL-4 treatment. In contrast, none of rats survived longer than 43 day after other treatment (9L-IL-12, 9L-IFNα or 9L-GM-CSF) or control treatment (9L-neo or HBSS) (11). Pericle et al. had a similar observation using spontaneous adenocarcinoma TS/A (6). They found TS/A-IL-4 elicited a more efficient protective immunity against subsequent parental tumor cell challenge than TS/A-IL-2 (6).

These observations seem to point out a way to cure cancer patients with IL-4. However, clinical outcomes were disappointing (12-14). In fact, cancer patients usually

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exhibited increased IL-4 level in tumor environment, and their lymphocytes, no matter peripheral blood lymphocytes or tumor infiltrating lymphocytes, were mostly polarized to secrete IL-4, as well as other Th2 cytokines. Shurin et al. summarized the data from plenty of clinical studies that investigated Th1/Th2 balance in cancer patients. They found IL-4, as well as other Th2 cytokines, was usually up-regulated in patients with different types of cancers, such as renal cell cancer, non-small lung cancer, prostate cancer, colon cancer, breast cancer and other types of tumors (15). Onishi et al. even found the IL-4 amount at tumor site was associated with the stage and grade of renal cancer (16).

The tumor-boosting effects of IL-4 were further confirmed by animal tumor models. EL4-B7-2, a murine lymphoma with the expression of costimulatory molecular B7-2, grew progressively in wild type mice, but was rejected by IL-4 knockout mice (17). LL-LCMV, a derivative of Lewis lung carcinoma that has been modified to express a minigene encoding LCMV33-41, exhibited delayed growth in IL-4 knockout (18). The evidence indicates IL-4 may provide some kind of help for tumor growth *in vivo*.

Even worse, IL-4 promotes tumor metastasis (19). Kobayashi et al. found the high metastatic variant of B16 melanoma (B16F10) induced a $CD4^+$ T cell population with strong IL-4-producing ability compared with T cells induced by the low metastatic variant, B16F1. Systemic IL-4 administration increased the metastasis of B16F1 to the level of B16F10. Neutralization of IL-4 using IL-4 specific mAb decreased the metastasis of B16F10 to the level of B16F1 (19).

How can IL-4 have opposite effects on tumor growth? It is really an interesting question.

Exogenous and endogenous IL-4

According to the sources, IL-4 utilized by the above studies can be divided into two groups, exogenous and endogenous IL-4. Endogenous IL-4 refers to the IL-4 molecules that are generated without human intervention in a physiological or pathological process. Endogenous IL-4 is usually produced by T cells, basophiles and mast cells and its production is strictly regulated by various interacting signals (20, 21). The facts, such as which cells produce, when and how much to produce, are mostly unknown now, but presumably should be carefully orchestrated to achieve homeostasis of immune system. In contrast, exogenous IL-4 is either systemically delivered as recombinant protein into the hosts (12, 14) or expressed locally by gene-modified tumor cells (3). In gene-modified tumor cells, the promoters controlling IL-4 expression are usually strong viral promoters or promoters of the house keeping genes, which lead to persistent IL-4 production in copious amount. So, in the situation implanting IL-4-gene-modified tumor cells into hosts, tumor site would have large amount of IL-4 from the moment tumor begins to grow.

Besides, the cells producing endogenous IL-4 are mobile and they may secret IL-4 both in the tumor site and in lymph nodes where the antigen presentation takes place. In contrast, for IL-4-gene-modified tumors, IL-4 production is restricted at tumor site. Analyzing the roles of endogenous IL-4 will help us to understand the physiological role of this cytokine during tumor development, whereas analyzing the roles of exogenous IL-4 is useful for developing novel strategies for tumor therapy.

By summarizing the available studies to date, we find the positive, or negative roles of IL-4 in tumor immunity are closely associated with its sources, with endogenous IL-4 promoting, while exogenous IL-4 often suppressing tumor growth. In the following parts, we will review the actions of IL-4 on various types of cells in tumor environment and the underlying mechanisms of IL-4 promoting or suppressing tumor growth. Hereafter, exogenous IL-4 is specifically referred to IL-4 produced by genetically modified tumor cells.

The mechanism of exogenous IL-4-induced tumor rejection

In early studies performed by Tepper et al., eosinophils were thought to be the primary effectors responsible for tumor rejection (3). This type of cells, as well as macrophages, existed in abundant amount in IL-4 secreting tumor site. Depletion of eosinophils using the mAb RB6-8C5 (Anti- Gr1, a myeloid differentiation antigen) restored the growth of IL-4-secreting tumor in BALB/c mice (22). However, the idea of considering eosinophils as the primary effectors encountered a little trouble when people found that tumor cells engineered to secrete IL-5 induced abundant eosinophil infiltration, but could not be rejected (23). In fact, besides eosinophils, neutrophils were also present at IL-4-producing tumor site (6). The antigen recognized by RB6-8C5 is expressed on both eosinophils and neutrophils (22). In the cell-depletion experiments performed by Tepper, RB6-8C5 treatment simultaneously depleted eosinophils and neutrophils. It cannot be excluded that eosinophils were just by-standers, while neutrophils were the true effectors. This idea was eventually substantiated in 1993 by Noffz et al. (9). They found eosinophil-deficient $IL\text{-}5^{-/-}$ mice exhibited similar ability to suppress IL-4-secreting tumor as wild type mice. In these mice, no eosinophils, but many neutrophils were detected in tumor mass. Depletion of these neutrophils using RB6-8C5 partly restored tumor growth (9).

The recognition of T cell contribution in exogenous IL-4-induced tumor rejection is also an uneven way. Initial studies indicated that T cells, as well as B cells, NK cells and mast cells, were not required for the IL-4 induced protective immunity, because no tumor grew out in nu/nu (deficient of T cells), SCID (deficient of T and B cells), bg/bg (deficient of NK cells), w/w^v (deficient of mast cells) mice that received *s.c.* inoculation of IL-4-producing plasmacytoma, J558L or melanoma B16 cells (22). However, also using IL-4 producing plastocytoma J558L, Hock et al. had a different observation. They observed the growth of this tumor in T cell-deficient mice, including nu/nu, SCID/beige (deficient of

T, B and NK cells) and NIH III mice (also deficient of T, B and NK cells) (24). Nonetheless, a long latency (about 30 days) existed before tumor nodule became palpable in these immuno-compromised mice. In immuno-competent mice, no tumor grew out. Cell depletion study indicated that $CD8^+$ T lymphocyte were important, whereas $CD4^+$ T cells played only a marginal role (24). At present, a biphasic mechanism is considered to be operating for exogenous IL-4-induced tumor rejection during which a rapid, innate immune cell dominant inflammatory response inhibited tumor burden, whereby allowing T cells to be activated and to finally complete tumor rejection (Figure 1).

Besides immune cells, IL-4 also acts on endothelial cells, inhibiting tumor-induced vascularization and starving tumor cells. Basic fibroblast growth factor (bFGF) is a potent inducer of angiogenesis and mounted a vigorous angiogenic response when implanted into the avascular cornea of rats as hydron pellet (25). However, the angiogenesis was completely blocked by IL-4. When anti-IL-4 mAb was added, new blood vessels formed again (25). *In vitro*, IL-4 inhibited the migration of cultured bovine and human microvascular cells and suppressed the formation of tube-like structure by human umbilical vein endothelial cells (HUVEC) induced by vascular endothelial growth factor (VEGF) and bFGF (26). Moreover, Saleh et al. observed that IL-4-induced inhibition of C6 rat glioma was accompanied by reduced levels of vascularization (27, 28). Practically, multiple factors, including innate immune cells, T lymphocytes and angiostasis may contribute to tumor rejection mediated by exogenous IL-4.

Endogenous IL-4 deviates host immune response to an ineffective status

It is well known that according to the cytokine profiles, $CD4^+$ T cells are divided into Th1, which produces IFNγ, IL-2 and tumor necrosis factor beta (TNFβ), and Th2, which produces IL-4, IL-5, IL-6, IL-9 and IL-13. These two types of T cells are cross-inhibitory. IFNγ and IL-12 promote the generation of Th1 cells and inhibit Th2 cell development. Contrarily, IL-4 drives the development of Th2 cells and inhibits Th1 response (29, 30).

Since the discovery of Th1 and Th2 cells by Mosmann in 1986 (31), their impacts on infection, allergy, autoimmune disease and transplantation etc have been extensively studied (15). In tumor immunity, Th2-dorminant immune response often fails to protect hosts from tumor growth. Lee et al. inoculated B-cell lymphoma (BCL1) into major histocompatibility complex (MHC)-compatible susceptible hosts, BALB/c mice ($H\text{-}2^d$), and MHC-compatible resistant hosts, B10.D2 mice ($H\text{-}2^d$). A Th2-dorminant immune response was elicited in BALB/c mice and the mice died within approximate 4 weeks. Whereas, Th1-dorminant immune response was elicited in B10.D2 mice and tumors were rejected (32). Hu et al. got the same conclusion using melanoma D5 and its allogeneic MHC class I gene ($H\text{-}2K^d$) modified variant, D5-Kd as tumor vaccines. Although both of them induced T cell expansion in the draining lymph nodes (DLNs), only T cells from DLNs of D5-Kd-immunized mice have therapeutic effects upon adoptive transfer. When

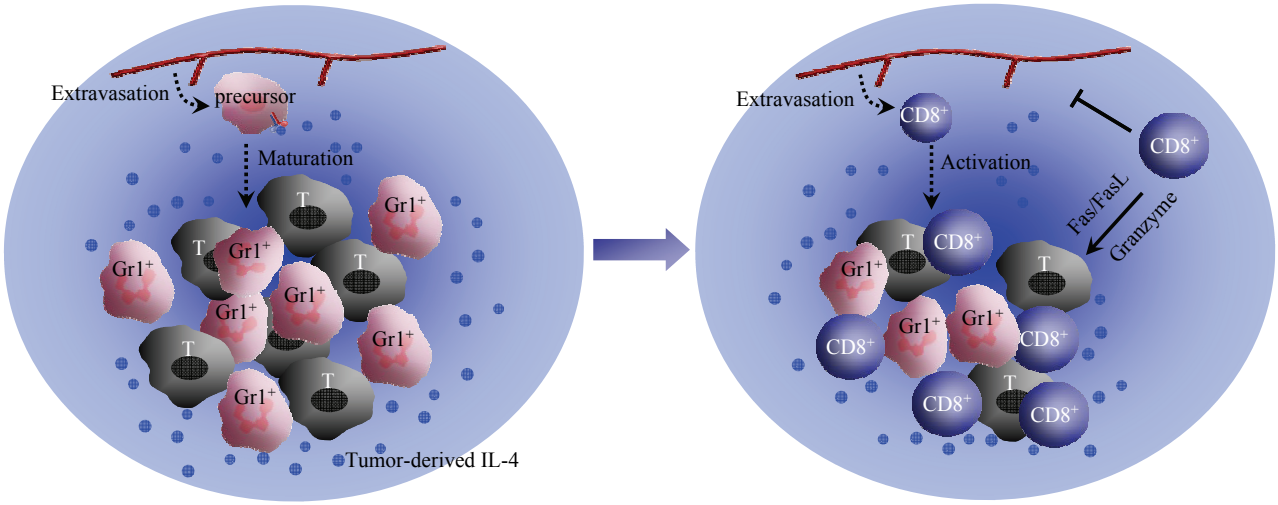

Figure 1. Tumor cell derived IL-4 induces tumor rejection. A possible gradient of concentration of exogenous IL-4 around the gene-modified tumor cells (T) is illustrated. IL-4 induces accumulation of myeloid precursor cells and promotes their maturation to Gr-1^+ granulocytes ($Gr1^+$). These granulocytes directly inhibit tumor cell expansion (Phase I), while $CD8^+$ T cells ($CD8^+$), activated in the presence of high levels of exogenous IL-4, are responsible for complete tumor rejection (Phase II). IL-4 directly, or through innate and acquired immune cells, inhibits tumor-induced angiogenesis.

stimulated *in vitro*, these T cells produced IFNγ, while T cells from the DLNs of D5 tumor immunized mice produced more IL-4 and IL-10 (33).

The failure of a Th2-dorminant immune response to protect hosts may be ascribed to the effects of IL-4 on $CD8^+$ T cells. $CD8^+$ T cells express MHC-I and kill target cells by Fas-FasL or perforin/granzyme pathway. Although not always, tumor clearance mostly relies on $CD8^+$ T cells. As early as in 1993, IL-4 was found to induce ionomycin/phorbol myristate acetate (PMA) stimulated $CD8^+$ T cells to differentiate into non-lytic $CD4^-CD8^-$ T cells (34). Kienzle et al. also demonstrated that culturing naïve murine $CD8^+$ T cells in the presence of IL-4 and anti-IFNγ mAb led to the generation of $CD8^{low}$ cells that were poorly cytolytic and expressed low levels of perforin and granzyme A, B and C (35, 36).

It is possible that the endogenous IL-4, on one side, acts on $CD4^+$ T cells to stabilize the Th2 status, on the other side, acts on $CD8^+$ T cells to render them non-cytotoxic (Figure 2). Of course, other mechanisms may also have some contributions, such as IL-10, which is a Th2 cytokine and induced by IL-4 and has been shown to have suppressive effects on tumor immunity.

However, things are often not so simple. Some studies have shown that IL-4-induced $CD8^+$ Tc2 cells still remain, sometimes even have increased cytotoxicity (37-40). So, the effects of IL-4 on $CD8^+$ T cells may rely on the initial cell status, the presence of other cytokines and the stimulating methods.

Tumor cells express elevated amount of IL-4R

Besides regulating local immune responses, IL-4 seems to exert direct effects on tumor cells. This suspicion arises from the observation that many types of tumor cells have elevated amount of IL-4R on their surface. Puri group did extensive studies on the amount, affinity and structure of IL-4R on tumor cells. Using I^{125}-IL-4 binding assay, combined with RT-PCR, northern-blotting and FACS, they measured the expression and affinity of IL-4R on murine sarcoma (41), human renal cell carcinoma (42), human AIDS-associated Kaposi sarcoma (43, 44), human lung cancer (45), ovarian cancer (46), Hodgkin lymphoma (47), breast carcinoma (48, 49), head and neck cancer (50, 51), pancreatic cancer (52) and other cancers. In 2001, they reviewed part of these studies (53). Although variation exists among different types of tumors, tumor cells overall have high amount of IL-4R, usually > 1000 of IL-4 binding sites/cell, sometimes reaches 4000 (renal cell carcinoma and breast cancer) and even > 10000 in head and neck cancer. In contrast, normal cells, even the cells on which IL-4 has profound effects, express much less IL-4R. For example, endothelial cells have < 50 IL-4 binding sites/cell, rest T cells and B cells have < 500, basophiles have 300-600 IL-4 binding sites/cell (53).

IL-4 binding affinity also exhibits large amount of differences between tumor and normal cells. On tumor cells, the dissociation constants (Kd) are usually > 100, and sometimes reach 1000 (breast cancer), while on normal cells, it is usually < 100 (53). To investigate IL-4R expression *in situ*, many immunohistological studies were done. In 12 normal and malignant specimens from human pancreas, only one of 5 normal pancreases showed weak to moderate positive staining for IL-4R. In contrast, all seven cancer specimens exhibited much more intensive staining (52). Fifty-four lung tumor samples were also analyzed for IL-4R expression. Between 66 and 79% samples were positive for IL-4R staining, whereas normal lung tissues only showed weak staining (45).

Why have tumor cells increased IL-4R expression? According to the immunosurveillance theory, tumor initiation and progression are accompanied by the attack from host immune system (54). To survive, tumor cells must keep changing to get abilities to break down various limitations inherent in cells or imposed by immune system (55). The elevated IL-4R expression may be one of such abilities obtained by tumor cells. Our group recently investigated the relationship of IL-4R expression and tumorigenic potential of tumor cells (56). To do this, we established primary fibrosarcoma FA61 by *s.c.* injecting carcinogen 3-methylcholanthrene (MCA) into BALB/c mice. The primary tumor cells had only weak ability to form tumors when injected into BALB/c mice. *In vivo* passage in immunocompetent mice increased their tumorigenic ability. Interestingly, IL-4R expression increased simultaneously. To demonstrate increased IL-4R expression indeed confer some growth advantages to tumor cells, we established a pair of tumor cells with or without IL-4R expression from the same parental $IL\text{-}4R^{-/-}$ tumor cells. When inoculated into wild type and IL-4R knockout mice, $IL\text{-}4R^+$ tumors exhibited increased growth than its $IL\text{-}4R^-$ counterpart. These studies indicate that IL-4R expression really has close relationship with tumorigenic potential of tumor cells.

IL-4 protects tumor cells from apoptosis

IL-4 has multiple effects on B cells and T cells, such as promoting immunoglobulin heavy chain switching to IgG1 and IgE (57), increasing the expression of MHC class II antigens (58), low-affinity Fc receptor (CD23) (59), surface IgM, cell adhesion molecules (LFA-1/LFA-3) (20) and facilitating Th2 development (60). However, besides these effects, IL-4 also protects B cells and T cells from apoptosis. Illera et al. observed that the addition of IL-4 significantly reduced spontaneous apoptosis of small dense B cells from mouse spleen (61). One year later, Parry et al. demonstrated that IL-4 prevented mature B cells from apoptosis induced by hyperlinking surface IgM or IgD receptor (62). Then, Wagner et al. found naïve B cells expressed higher level of IL-4R than germinal center and memory B cells. When culturing *in vitro*, IL-4 protected naïve B cells, but not germinal center or memory B cells from apoptosis (63). Similar results were found in T cells (64-66). IL-4 reduced the susceptibility of human $CD8^+$ T cells to activation-induced cell death by maintaining the level of survival-related protein Bcl-2 (65).

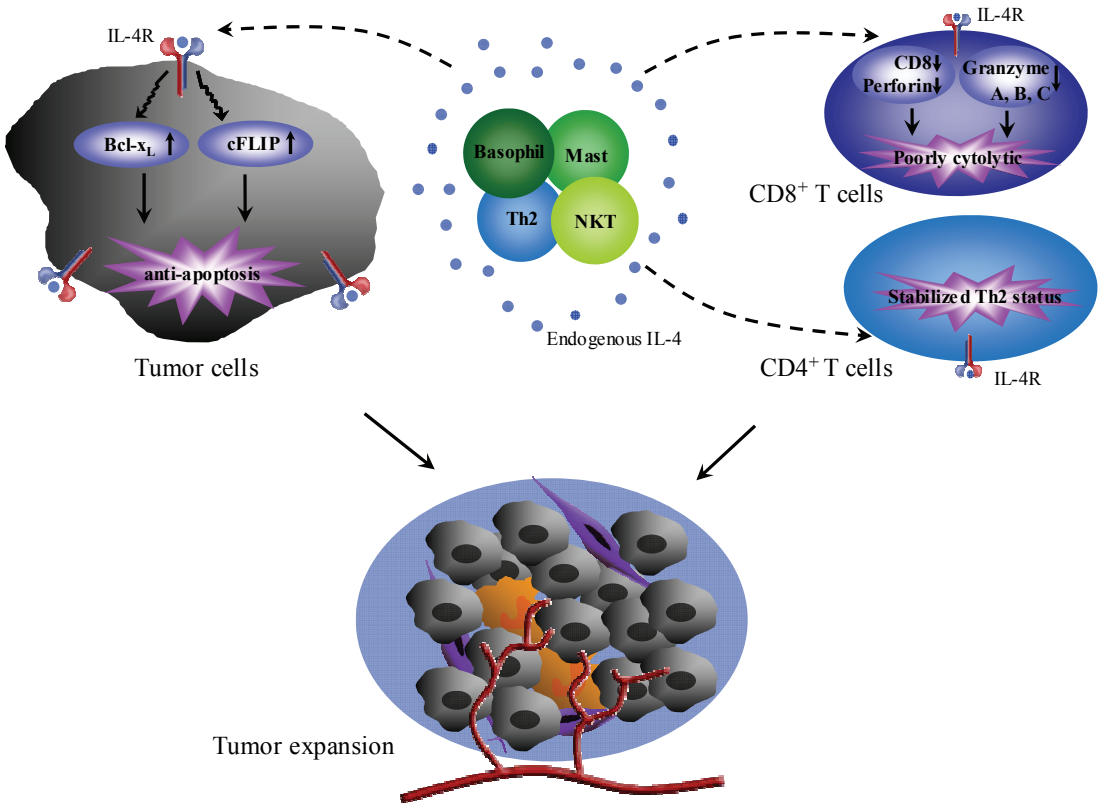

Figure 2. Endogenous IL-4 promotes tumor growth. The timing and concentration of endogenous IL-4 expression in tumor site are almost unknown. Many host cells, including Th2 cells, basophils, mast cells and NKT cells, can secret IL-4 under a proper stimulation. Endogenous IL-4 acts on $CD4^+$ T cells, inducing and stabilizing their Th2 status. IL-4 also down-regulates the expression of CD8, perforin, and granzyme A, B, and C in $CD8^+$ T cells and commits these cells to a poorly cytolytic phenotype. Furthermore, tumor cells often have increased IL-4R expression. Binding of IL-4 to its receptor on tumor cells increases the level of anti-apoptosis molecules, such as Bcl-x_L and cFLIP. These multiple effects of endogenous IL-4 together lead to immune deviation and tumor expansion.

With these observations in normal B/T cells, it is easy to imagine that IL-4 promotes the survival of B/T cell-derived tumors. As early as in 1992, Danceca reported that IL-4 inhibited spontaneous and hydrocortisone-induced cell death of malignant B cells purified from patients with B chronic lymphocytic leukemia (67).

Besides immune cells, IL-4 also protects other types of cells from apoptosis. Thyrocytes from patients with Grave diseases, a type of hyperthyroidism characterized by increased vascularization and enlargement of thyroid, express both CD95 and CD95L (68, 69). But these cells are resistant to anti-CD95 monoclonal antibody-induced apoptosis. Long-term culture (4 days) sensitized them to apoptosis again, accompanied by the disappearance of cellular Bcl-x_L and cFLIP. IL-4 presence in the long-term culture maintained Bcl-x_L and cFLIP level and led to resistance of thyrocytes to apoptosis (68).

Thyroid cancer is a type of malignancy arisen from thyroid epithelial. Stassi et al. demonstrated thyroid cancer cells showed refractoriness to chemotherapeutic agents including cisplatin, doxorubicin and taxol treatment (70). Todaro found cancer cells from papillary, follicular or undifferentiated anaplastic thyroid carcinoma were resistant to CD95-induced apoptosis (71). All these effects were IL-4-dependent (70, 71). Other cancer cells, such as human bladder cancer cell RT112, prostate cancer cell LNCap and breast cancer cell MDA-MB-231 also exhibited IL-4-dependent apoptosis resistance (72). In the presence of IL-4, these cells expressed high levels of cellular Bcl-x_L, cFLIP or both of them. The investigators in our group observed the similar effects of IL-4 on murine fibrosarcoma (56), and using IL-4R competent or deficient tumor cells of the same origin demonstrated that IL-4 promotes tumor growth *in vivo* (Figure 2).

The expression time and dose may determine the effects of IL-4 on tumor growth

Why can the same molecule have opposite effects on tumor growth? Maybe the different expression patterns, including dose and time, result in the different effects. Actually, opposite effects of low and high dose IL-4 had been demonstrated by Volpert (25). They studied the migration of bovine adrenal capillary endothelial cells and human dermal capillary endothelial cells in the presence of a wide range

(10^{-3} - 10^{2} ng/ml) IL-4. The effects of IL-4 exhibited a biphasic modality with low concentration (10^{-3} - 10^{-2} ng/ml) promoting and high concentration (10^{-1} - 10^{2} ng/ml) inhibiting endothelial cell migration (25).

Recently, our group is studying the time and dose effects of IL-4 expression on tumor growth. We inserted IL-4 cDNA into the downstream of a Tet-regulated promoter and transfected the fibrosarcom MCA205 cells with this IL-4 expressing plasmid. IL-4 expression by MCA205 tumor cells is easy to be regulated by adding Tet to the culturing medium (*in vitro*) or by supplying mice with drinking water containing different concentrations of Tet (*in vivo*). Our data showed clearly that IL-4-mediated tumor rejection is strictly dose-dependent. Furthermore, we found early IL-4 expression was able to (before day 5 after tumor cell inoculation), but late IL-4 expression failed to suppress the growth of *s.c.* inoculated tumors. Abundant granulocyte infiltrations were observed in both tumor nodules with early or late IL-4 expression. However, the granulocytes in mice with late IL-4 expression had cell surface markers characteristic for myeloid-derived suppressor cells (MDSC), a cell population with tumor-promoting effects, whereas in mice with early IL-4 expression, they appear to be mature granulocytes (manuscript submitted).

Conclusions and future directions

Physiologically, IL-4 acts directly on tumor cells as a tumor promoting cytokine. It also contributes to the establishment and maintenance of Th2-polarized immune responses, reduces the tumoricidal activity of $CD8^+$ T cells and indirectly impairs the antitumor immunity in tumor bearing animals or cancer patients. However, it is still possible to make IL-4 as a useful cytokine for tumor therapy. The anti-tumor effects of IL-4 have been demonstrated in many types of tumors which were genetically modified to secrete IL-4. The mechanism may lie in the effect of IL-4 on maturation of myeloid precursor cells and therefore, more efficient priming of T cells.

Undoubtedly, there are still lots of questions open in the field of IL-4 and tumor immunity. For example, where does IL-4 come from during tumor development? How about the kinetics of IL-4 production in tumor microenvironment? What cell types does IL-4 exert its effects on and what is the specific consequence? Does IL-4 play similar roles in different tumors and in different species? Can we eventually use IL-4 as an adjuvant for tumor vaccines? Just like TNFα and many other cytokines, IL-4 is clearly a double-edged sword for the tumor. What we should do is to utilize the beneficial and avoid the deleterious effects.

Acknowledgements

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References

1. Lee F, Yokota T, Otsuka T, et al. Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 activities and T-cell- and mast-cell-stimulating activities. Proc Natl Acad Sci U S A. 1986;83:2061-2065.
2. Noma Y, Sideras P, Naito T, et al. Cloning of cDNA encoding the murine IgG1 induction factor by a novel strategy using SP6 promoter. Nature. 1986;319:640-646.
3. Tepper RI, Pattengale PK, Leder P. Murine interleukin-4 displays potent anti-tumor activity *in vivo*. Cell. 1989;57:503-512.
4. Golumbek PT, Lazenby AJ, Levitsky HI, et al. Treatment of established renal cancer by tumor cells engineered to secrete interleukin-4. Science. 1991;254:713-716.
5. Pippin BA, Rosenstein M, Jacob WF, Chiang Y, Lotze MT. Local IL-4 delivery enhances immune reactivity to murine tumors: gene therapy in combination with IL-2. Cancer Gene Ther. 1994;1:35-42.
6. Pericle F, Giovarelli M, Colombo MP, et al. An efficient Th2-type memory follows $CD8^+$ lymphocyte-driven and eosinophil-mediated rejection of a spontaneous mouse mammary adenocarcinoma engineered to release IL-4. J Immunol. 1994;153:5659-5673.
7. Stoppacciaro A, Paglia P, Lombardi L, Parmiani G, Baroni C, Colombo MP. Genetic modification of a carcinoma with the IL-4 gene increases the influx of dendritic cells relative to other cytokines. Eur J Immunol. 1997;27:2375-2382.
8. Rodolfo M, Zilocchi C, Accornero P, Cappetti B, Arioli I, Colombo MP. IL-4-transduced tumor cell vaccine induces immunoregulatory type 2 CD8 T lymphocytes that cure lung metastases upon adoptive transfer. J Immunol. 1999;163:1923-1928.
9. Noffz G, Qin Z, Kopf M, Blankenstein T. Neutrophils but not eosinophils are involved in growth suppression of IL-4-secreting tumors. J Immunol. 1998;160:345-350.
10. Eguchi J, Kuwashima N, Hatano M, et al. IL-4-transfected tumor cell vaccines activate tumor-infiltrating dendritic cells and promote type-1 immunity. J Immunol. 2005;174:7194-7201.
11. Okada H, Villa L, Attanucci J, et al. Cytokine gene therapy of gliomas: effective induction of therapeutic immunity to intracranial tumors by peripheral immunization with interleukin-4 transduced glioma cells. Gene Ther. 2001;8:1157-1166.
12. Atkins MB, Vachino G, Tilg HJ, et al. Phase I evaluation of thrice-daily intravenous bolus interleukin-4 in patients with refractory malignancy. J Clin Oncol. 1992;10:1802-1809.
13. Gilleece MH, Scarffe JH, Ghosh A, et al. Recombinant human interleukin 4 (IL-4) given as daily subcutaneous injections--a phase I dose toxicity trial. Br J Cancer. 1992;66:204-210.
14. Stadler WM, Rybak ME, Vogelzang NJ. A phase II study of subcutaneous recombinant human interleukin-4 in metastatic renal cell carcinoma. Cancer. 1995;76:1629-1633.
15. Shurin MR, Lu L, Kalinski P, Stewart-Akers AM, Lotze MT. Th1/Th2 balance in cancer, transplantation and pregnancy. Springer Semin Immunopathol. 1999;21:339-359.
16. Onishi T, Ohishi Y, Imagawa K, Ohmoto Y, Murata K. An assessment of the immunological environment based on intratumoral cytokine production in renal cell carcinoma. BJU Int. 1999;83:488-492.

17. Stremmel C, Greenfield EA, Howard E, Freeman GJ, Kuchroo VK. B7-2 expressed on EL4 lymphoma suppresses antitumor immunity by an interleukin 4-dependent mechanism. J Exp Med. 1999;189:919-930.
18. Kemp RA, Ronchese F. Tumor-specific Tc1, but not Tc2, cells deliver protective antitumor immunity. J Immunol. 2001;167: 6497-6502.
19. Kobayashi M, Kobayashi H, Pollard RB, Suzuki F. A pathogenic role of Th2 cells and their cytokine products on the pulmonary metastasis of murine B16 melanoma. J Immunol. 1998;160:5869-5873.
20. Okada H, Kuwashima N. Gene therapy and biologic therapy with interleukin-4. Curr Gene Ther. 2002;2:437-450.
21. Nelms K, Keegan AD, Zamorano J, Ryan JJ, Paul WE. The IL-4 receptor: signaling mechanisms and biologic functions. Annu Rev Immunol. 1999;17:701-738.
22. Tepper RI, Coffman RL, Leder P. An eosinophil-dependent mechanism for the antitumor effect of interleukin-4. Science. 1992;257:548-551.
23. Kruger-Krasagakes S, Li W, Richter G, Diamantstein T, Blankenstein T. Eosinophils infiltrating interleukin-5 gene-transfected tumors do not suppress tumor growth. Eur J Immunol. 1993;23:992-995.
24. Hock H, Dorsch M, Kunzendorf U, Qin Z, Diamantstein T, Blankenstein T. Mechanisms of rejection induced by tumor cell-targeted gene transfer of interleukin 2, interleukin 4, interleukin 7, tumor necrosis factor, or interferon gamma. Proc Natl Acad Sci U S A. 1993;90:2774-2778.
25. Volpert OV, Fong T, Koch AE, et al. Inhibition of angiogenesis by interleukin 4. J Exp Med. 1998;188:1039-1046.
26. Lee IY, Kim J, Ko EM, Jeoung EJ, Kwon YG, Choe J. Interleukin-4 inhibits the vascular endothelial growth factor- and basic fibroblast growth factor-induced angiogenesis *in vitro*. Mol Cells. 2002;14:115-121.
27. Saleh M, Wiegmans A, Malone Q, Stylli SS, Kaye AH. Effect of *in situ* retroviral interleukin-4 transfer on established intracranial tumors. J Natl Cancer Inst. 1999;91:438-445.
28. Saleh M, Davis ID, Wilks AF. The paracrine role of tumour-derived mIL-4 on tumour-associated endothelium. Int J Cancer. 1997;72:664-672.
29. Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol. 1989;7:145-173.
30. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996;383:787-793.
31. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986;136:2348-2357.
32. Lee PP, Zeng D, McCaulay AE, et al. T helper 2-dominant antilymphoma immune response is associated with fatal outcome. Blood. 1997;90:1611-1617.
33. Hu HM, Urba WJ, Fox BA. Gene-modified tumor vaccine with therapeutic potential shifts tumor-specific T cell response from a type 2 to a type 1 cytokine profile. J Immunol. 1998;161: 3033-3041.
34. Erard F, Wild MT, Garcia-Sanz JA, Le Gros G. Switch of CD8 T cells to noncytolytic $CD8^-CD4^-$ cells that make TH2 cytokines and help B cells. Science. 1993;260:1802-1805.
35. Kienzle N, Buttigieg K, Groves P, Kawula T, Kelso A. A clonal culture system demonstrates that IL-4 induces a subpopulation of noncytolytic T cells with low CD8, perforin, and granzyme expression. J Immunol. 2002;168:1672-1681.
36. Kienzle N, Olver S, Buttigieg K, et al. Progressive differentiation and commitment of $CD8^+$ T cells to a poorly cytolytic CD8low phenotype in the presence of IL-4. J Immunol. 2005;174:2021-2029.
37. Widmer MB, Grabstein KH. Regulation of cytolytic T-lymphocyte generation by B-cell stimulatory factor. Nature. 1987;326:795-798.
38. Horohov DW, Crim JA, Smith PL, Siegel JP. IL-4 (B cell-stimulatory factor 1) regulates multiple aspects of influenza virus-specific cell-mediated immunity. J Immunol. 1988;141: 4217-4223.
39. Trenn G, Takayama H, Hu-Li J, Paul WE, Sitkovsky MV. B cell stimulatory factor 1 (IL-4) enhances the development of cytotoxic T cells from Lyt-2+ resting murine T lymphocytes. J Immunol. 1988;140:1101-1106.
40. Sad S, Marcotte R, Mosmann TR. Cytokine-induced differentiation of precursor mouse $CD8^+$ T cells into cytotoxic $CD8^+$ T cells secreting Th1 or Th2 cytokines. Immunity. 1995;2: 271-279.
41. Puri RK, Ogata M, Leland P, Feldman GM, FitzGerald D, Pastan I. Expression of high-affinity interleukin 4 receptors on murine sarcoma cells and receptor-mediated cytotoxicity of tumor cells to chimeric protein between interleukin 4 and Pseudomonas exotoxin. Cancer Res. 1991;51:3011-3017.
42. Obiri NI, Hillman GG, Haas GP, Sud S, Puri RK. Expression of high affinity interleukin-4 receptors on human renal cell carcinoma cells and inhibition of tumor cell growth *in vitro* by interleukin-4. J Clin Invest. 1993;91:88-93.
43. Husain SR, Gill P, Kreitman RJ, Pastan I, Puri RK. Interleukin-4 receptor expression on AIDS-associated Kaposi's sarcoma cells and their targeting by a chimeric protein comprised of circularly permuted interleukin-4 and Pseudomonas exotoxin. Mol Med. 1997;3:327-338.
44. Husain SR, Kreitman RJ, Pastan I, Puri RK. Interleukin-4 receptor-directed cytotoxin therapy of AIDS-associated Kaposi's sarcoma tumors in xenograft model. Nat Med. 1999;5:817-822.
45. Kawakami M, Kawakami K, Stepensky VA, et al. Interleukin 4 receptor on human lung cancer: a molecular target for cytotoxin therapy. Clin Cancer Res. 2002;8:3503-3511.
46. Kioi M, Takahashi S, Kawakami M, Kawakami K, Kreitman RJ, Puri RK. Expression and targeting of interleukin-4 receptor for primary and advanced ovarian cancer therapy. Cancer Res. 2005;65:8388-8396.
47. Kawakami M, Kawakami K, Kioi M, Leland P, Puri RK. Hodgkin lymphoma therapy with interleukin-4 receptor-directed cytotoxin in an infiltrating animal model. Blood. 2005;105: 3707-3713.
48. Leland P, Taguchi J, Husain SR, Kreitman RJ, Pastan I, Puri RK. Human breast carcinoma cells express type II IL-4 receptors and are sensitive to antitumor activity of a chimeric IL-4-Pseudomonas exotoxin fusion protein *in vitro* and *in vivo*. Mol Med. 2000;6:165-178.
49. Kawakami K, Kawakami M, Husain SR, Puri RK. Effect of interleukin (IL)-4 cytotoxin on breast tumor growth after *in vivo* gene transfer of IL-4 receptor α chain. Clin Cancer Res. 2003;9: 1826-1836.
50. Mehrotra R, Varricchio F, Husain SR, Puri RK. Head and neck cancers, but not benign lesions, express interleukin-4 receptors in situ. Oncol Rep. 1998;5:45-48.
51. Strome SE, Kawakami K, Alejandro D, et al. Interleukin 4 receptor-directed cytotoxin therapy for human head and neck squamous cell carcinoma in animal models. Clin Cancer Res. 2002;8:281-286.
52. Kawakami K, Kawakami M, Husain SR, Puri RK. Targeting interleukin-4 receptors for effective pancreatic cancer therapy.

Cancer Res. 2002;62:3575-3580.
53. Kawakami K, Kawakami M, Puri RK. Overexpressed cell surface interleukin-4 receptor molecules can be successfully targeted for antitumor cytotoxin therapy. Crit Rev Immunol. 2001;21:299-310.
54. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002;3:991-998.
55. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57-70.
56. Li Z, Jiang J, Wang Z, et al. Endogenous interleukin-4 promotes tumor development by increasing tumor cell resistance to apoptosis. Cancer Res. 2008;68:8687-8694.
57. Snapper CM, Finkelman FD, Paul WE. Differential regulation of IgG1 and IgE synthesis by interleukin 4. J Exp Med. 1988; 167:183-196.
58. Noelle R, Krammer PH, Ohara J, Uhr JW, Vitetta ES. Increased expression of Ia antigens on resting B cells: an additional role for B-cell growth factor. Proc Natl Acad Sci U S A. 1984;81: 6149-6153.
59. Defrance T, Aubry JP, Rousset F, et al. Human recombinant interleukin 4 induces Fc epsilon receptors (CD23) on normal human B lymphocytes. J Exp Med. 1987;165:1459-1467.
60. Seder RA, Paul WE, Davis MM, Fazekas de St Groth B. The presence of interleukin 4 during *in vitro* priming determines the lymphokine-producing potential of $CD4^+$ T cells from T cell receptor transgenic mice. J Exp Med. 1992;176:1091-1098.
61. Illera VA, Perandones CE, Stunz LL, Mower DA, Jr., Ashman RF. Apoptosis in splenic B lymphocytes. Regulation by protein kinase C and IL-4. J Immunol. 1993;151:2965-2973.
62. Parry SL, Hasbold J, Holman M, Klaus GG. Hypercross-linking surface IgM or IgD receptors on mature B cells induces apoptosis that is reversed by costimulation with IL-4 and anti-CD40. J Immunol. 1994;152:2821-2829.
63. Wagner EF, Hanna N, Fast LD, et al. Novel diversity in IL-4-mediated responses in resting human naïve B cells versus germinal center/memory B cells. J Immunol. 2000;165:5573-5579.
64. Vella A, Teague TK, Ihle J, Kappler J, Marrack P. Interleukin 4 (IL-4) or IL-7 prevents the death of resting T cells: stat6 is probably not required for the effect of IL-4. J Exp Med. 1997; 186:325-330.
65. Riou C, Dumont AR, Yassine-Diab B, Haddad EK, Sekaly RP. IL-4 influences the differentiation and the susceptibility to activation-induced cell death of human naive $CD8^+$ T cells. Int Immunol. 2006;18:827-835.
66. Rautajoki KJ, Marttila EM, Nyman TA, Lahesmaa R. Interleukin-4 inhibits caspase-3 by regulating several proteins in the Fas pathway during initial stages of human T helper 2 cell differentiation. Mol Cell Proteomics. 2007;6:238-251.
67. Dancescu M, Rubio-Trujillo M, Biron G, Bron D, Delespesse G, Sarfati M. Interleukin 4 protects chronic lymphocytic leukemic B cells from death by apoptosis and upregulates Bcl-2 expression. J Exp Med. 1992;176:1319-1326.
68. Stassi G, Di Liberto D, Todaro M, et al. Control of target cell survival in thyroid autoimmunity by T helper cytokines *via* regulation of apoptotic proteins. Nat Immunol. 2000;1:483-488.
69. Stassi G, De Maria R. Autoimmune thyroid disease: new models of cell death in autoimmunity. Nat Rev Immunol. 2002;2:195-204.
70. Stassi G, Todaro M, Zerilli M, et al. Thyroid cancer resistance to chemotherapeutic drugs *via* autocrine production of interleukin-4 and interleukin-10. Cancer Res. 2003;63:6784-6790.
71. Todaro M, Zerilli M, Ricci-Vitiani L, et al. Autocrine production of interleukin-4 and interleukin-10 is required for survival and growth of thyroid cancer cells. Cancer Res. 2006;66:1491-1499.
72. Conticello C, Pedini F, Zeuner A, et al. IL-4 protects tumor cells from anti-CD95 and chemotherapeutic agents via up-regulation of antiapoptotic proteins. J Immunol. 2004;172:5467-5477.