Interleukin-27 Activates Natural Killer Cells and Suppresses NK-Resistant Head and Neck Squamous Cell Carcinoma through Inducing Antibody-Dependent Cellular Cytotoxicity

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Abstract

Interleukin (IL)-27 is an IL-12 family cytokine playing a pivotal role in the induction of Th1 immune responses, although its action on natural killer (NK) cells has not been fully elucidated. Here, we show that IL-27 is capable of inducing phosphorylation of signal transducers and activators of transcription 1 and 3, as well as expression of T-bet and granzyme B in murine DX-5+ NK cells. IL-27 also enhances cytotoxic activity of NK cells both in vitro and in vivo, while the in vitro viability of NK cells is also improved by this cytokine. Therapeutic administration of the IL-27 gene drastically suppressed the growth of NK-unsusceptible SCCVII tumors that had been preestablished in syngenic mice, resulting in significant prolongation of the survival of the animals. This can likely be ascribed to the antibody-dependent cellular cytotoxicity machinery because IL-27 successfully induced tumor-specific IgG in the sera of the tumor-bearing mice, and supplementation of the sera enabled IL-27-activated NK cells to kill SCCVII cells in an Fcy receptor III-dependent manner. These findings strongly suggest that IL-27 may offer a powerful immunotherapeutic tool to eradicate head and neck squamous cell carcinoma and other poorly immunogenic neoplasms through activating NK cells and inducing tumor-specific immunoglobulin that may cooperatively elicit antibody-dependent cellular cytotoxicity activity.

Introduction

Recent advances in therapeutic procedures including surgical operation appreciably improved the 5-year survival of patients with the head and neck squamous cell carcinoma (HNSCC). However, surgical management frequently causes serious functional problems including vocal, articulatory, swallowing and chewing disorders, as well as cosmetic defects, while inoperable advanced HNSCC is usually resistant to conventional radiotheraphies and chemotherapies. Cytokine immunotherapy may provide promising nonsurgical therapeutic modalities to concur some highly immunogenic malignancies such as melanoma and renal cell carcinoma, by virtue of the induction of specific and/or nonspecific effectors that attack tumor cells. However, HNSCC is one type of tumor that is relatively resistant to conventional immunotherapeutic interventions because it is usually unsusceptible to natural killer (NK) cytolysis, while cytotoxic T lymphocytes (CTLs) specific for HNSCC are hard to induce. In this context, a novel cytokine that induces powerful antitumor effector mechanisms is desired to improve the cure rate as well as the quality-of-life of HNSCC patients.

Interleukin (IL)-27 is a member of the IL-12 cytokine family, consisting of heterodimeric polypeptides, EBV-induced gene 3 (EBI3) protein, and p28, which are homologous to the p40 and p35 subunits of IL-12, respectively (1). IL-27 is mainly produced by activated antigen-presenting cells including lipopolysaccharide-stimulated monocyte and monocyte-derived dendritic cells (1). The IL-27 receptor (IL-27R) complex comprises two polypeptide chains, i.e., IL-27RA (also called WSX-1 or TCCR) and gp130 (1, 2). The former is specific for IL-27, while the latter is shared by the receptor complexes for IL-6, IL-11, CNTF, LIF, CLC, OSM, and CT-1. The gp130 chain plays essential roles in IL-27 signal transmission (2), in which Jak1 and signal transducers and activators of transcription (STAT)1 are consecutively activated (3). Finally, STAT3 and 5 may also be involved in the IL-27 signaling (4–6).

IL-27 exerts pleiotrophic immunomodulatory effects on a broad spectrum of immune cells. When naive CD4+ T cells are stimulated by IL-27 in vitro, the cytokine induces the Th1-associated transcription factor T-bet as well as its downstream target IL-12Rβ2, leading to proliferation and Th1 polarization of the naive CD4+ T cells (3, 5, 6). IL-27 also synergizes with IL-12 for production of the typical Th1 cytokine, IFN-γ (1, 3). In contrast, the IL-27 signal prevents differentiation of Th2, Th17, and Treg cells through suppressing the corresponding key transcription factors, i.e., GATA-3, ROR-γt, and Foxp3, respectively (6–9). Naïve CD8+ T cells are also provoked by IL-27 to express T-bet, IL-12Rβ2, and granzyme B (10) to facilitate the generation and proliferation of CTL (10–12). IL-27 modulates B-cell responses depending on the differentiation stages and activation statuses (13, 14), while possibly exerting negative effects on NKT cells, another distinct lymphoid cell population (15, 16). Nonlymphoid cells including macrophages (17), dendritic cells (18), and mast cells (19) are also influenced by this cytokine (19).

As for the direct action of IL-27 on NK cells, Pfanz and colleagues (1) showed that IL-27 ligation enhances T-bet expression in human NK cells. The cytokine signal significantly increases IFN-γ production by NK cells that are stimulated by both IL-2 and IL-12, whereas IL-27 per se fails to provoke NK cells to produce IFN-γ, and IL-27 elevates neither IL-2–induced nor IL-12–induced IFN-γ production by NK cells (1). More profound investigation of...
IL-27 activity on NK cells may provide useful information to devise a novel immunotherapeutic approach against tumors, in which this cytokine is applied as the therapeutic tool to activate NK cells as effector cells.

In the present study, we examined the regulatory activity of IL-27 on murine NK cells. Moreover, we investigated the therapeutic potential of IL-27 against s.c. HNSCC in mice, and analyzed the immunologic basis of the antitumor responses, including the

Figure 1. IL-27 signaling in NK cells. A, splenic DX-5+CD4−CD8−B220− cells were obtained from C3H/HeN mice and stimulated with 100 ng/mL rmIL-27 for 20 min. Cells were then lysed and subjected to Western blotting and probed with the indicated antibody. B, DX-5+CD4−CD8−B220− cells were cultured with 100 ng/mL rmIL-27 for the indicated periods. RNA was extracted and subjected to real-time RT-PCR to evaluate T-bet (left) and granzyme B (right) mRNA. C, DX-5+CD4−CD8−B220− cells were cultured in the presence or absence of 100 ng/mL rmIL-27, 5 ng/mL anti–IFN-γ neutralizing antibody, and 20 ng/mL soluble WSX-1 as indicated. Eighteen hours later, T-bet (left) and granzyme B (right) mRNA was evaluated as above. Columns, mean of triplicate samples are shown; bars, SE. *, P < 0.05. ND, not determined.

Figure 2. IL-27 enhances killer activity and viability of NK cells. A, splenic DX5+ cells were obtained from SCCVII tumor–bearing mice and cultured with 0, 10, or 100 ng/mL rmIL-27. Eight hours later, cells were subjected to 51Cr release assay using YAC-1 cells as a target. Points, mean of triplicate samples; bars, SD. *, P < 0.05, at E/T of 40:1. B, splenic DX5+ cells obtained from tumor-bearing mice were cultured with rmIL-27 or rmIL-2. At indicated hours later, viable cells were enumerated. Points, mean of triplicate samples; bars, SD. *, P < 0.05, at 48 h. C, SCCVII tumor–bearing mice were transfected with the indicated plasmid or left untreated as a control. Forty-eight hours later, splenic DX5+ cells were collected and subjected to 51Cr release assay using YAC-1 cells as a target. n = 3 mice. *, P < 0.05, at E/T of 100:1. D, tumor bearers were transfected with the indicated plasmid, or left untreated (control). At indicated hours later, sera were collected and concentrations of IFN-γ were measured by ELISA. Columns, mean (n = 5 mice); bars, SD. *, P < 0.05, at 20 h.
contribution of NK cells and immunoglobulin-mediated attack of the cancer cells.

Materials and Methods

Animals and cell preparation. All the animal experiments were performed according to the approved guidelines of Kyoto Prefectural University of Medicine. Six- to eight-week-old female C3H/HeN mice were purchased from Shimizu Laboratory Suppliers Co. Ltd. Splenic DX-5 cells were enriched using magnetic beads, whereas DX-5+CD4+CD8+cells were enriched using CD4, CD8, anti-B220, and anti-DX antibodies in combination with magnetic beads (Miltenyi Biotec). More than 95% of the population was DX5+ as confirmed by flow cytometric analysis.

Recombinant cytokines and reagents. Recombinant mouse IL-27 (rmIL-27), rmIL-2, and soluble WSX-1 were purchased from R & D Systems. Recombinant mouse IL-27 protein Prep kit (Qiagen). Each aliquot of the cell extract containing 30 μg of soluble protein was electrophoresed through a 10% SDS polyacrylamide gel, which was subsequently blotted onto a polyvinylidene difluoride membrane. After probing with antibodies that recognize STAT1, phospho STAT1 (pY701), STAT3, and phospho STAT3 (pS727; BD Biosciences), the signals were visualized using chemiluminescent Western blot immunodetection kit (Invitrogen).

Flowcytometric analyses. To assess tumor-specific IgG in the mouse serum, SCCVII and C2C12 cells were incubated with diluted sera for 20 min on ice. After washing twice, the cells were incubated with phycoerythrin-conjugated rabbit anti-mouse IgG (Zymed), followed by further washing on ice. After washing twice, the cells were incubated with phycoerythrin-serum, SCCVII and C2C12 cells were incubated with diluted sera for 20 min with anti-asialo GM1 antibody. Points, means of the volumes of tumors (A and C) as well as survival rates of animals (B and D); bars, SD. n = 6 (A and B) or 7 (C and D) mice in each group. * P < 0.05, on days 27 (A), 42 (C), and 80 (B and D).

Figure 3. IL-27 gene transfer drastically suppresses SCCVII tumors and prolongs longevity of the tumor bearer. Mice received s.c. inoculation of 1 × 106 SCCVII cells into the flank (day 0). On day 5, the indicated plasmid was i.v. transfected into mice at a dose of 20 μg and the treatment was repeated every 7 d (arrows). A group of tumor-bearing mice were left untreated as a control. In C and D, a group of tumor-bearing mice also received i.p. administrations of anti-asialo GM1 antibody. Points, means of the volumes of tumors (A and C) as well as survival rates of animals (B and D); bars, SD. n = 6 (A and B) or 7 (C and D) mice in each group. * P < 0.05, on days 27 (A), 42 (C), and 80 (B and D).

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obtained from C3H/HeN mice expressed WSX-1 as well as gp130 on their surface (data not shown). Then we examined signal transduction in C3H/HeN splenic DX-5+CD4+CD8- B220- cells after IL-27 stimulation. As shown in Fig. 1A, the cytokine induced phosphorylation of STAT1 and 3 at tyrosine 701 and serine 727, respectively. The IL-27 signal also induced T-bet and granzyme B mRNA that were previously shown to be induced in CD4+ and CD8+ T cells (Fig. 1B; ref. 10). The T-bet and granzyme B mRNA levels reached their maximal levels at 8 hours after the addition of rmIL-27 (Fig. 1B). These effects were totally abrogated by the addition of soluble WSX-1 that competitively blocks IL-27-IL-27RA interaction, clearly demonstrating that IL-27-R was crucially involved in the events (Fig. 1C). The T-bet and granzyme B expression was not secondarily induced by IFN-γ that could potentially be produced by IL-27-stimulated NK cells because anti–IFN-γ neutralizing antibody failed to interfere with the induction of these genes (Fig. 1C). These results indicated that IL-27 directly stimulates NK cells through a functional IL-27R complex.

IL-27 positively regulates viability and cytolytic activity of NK cells. Next, we examined whether IL-27 influences killing activity and/or survival of NK cells. Splenic DX-5+ cells were obtained from tumor-bearing mice that had been established by a s.c. inoculation of the HNSCC cell line SCCVII into syngenic mice, and the splenic NK cells were cultured with rmIL-27 at concentrations of 10 or 100 ng/mL. As shown in Fig. 2A, 51Cr release assay showed that 100 ng/mL of rmIL-27 remarkably augmented the cytotoxicity against NK-sensitive YAC-1 cells at every E/T ratio tested, but the NK activity was not significantly affected by rmIL-27 at 10 ng/mL.

A significant proportion of NK cells died in culture in the absence of any cytokine stimulation (Fig. 2B). It was also found that addition of 100 ng/mL of rmIL-27 promoted viability of NK cells more significantly than addition of rmIL-2- at the same dose (Fig. 2B). The results showed that IL-27 signal elevated viability of NK cells in culture, although the proliferation of the cells may not be significantly and continuously promoted by the cytokine.

We also estimated the functional modification in vivo of NK cells by IL-27. Mice bearing a SCCVII tumor were transfected with an IL-27 expression plasmid by means of the hydrodynamics-based procedure (21). The procedure resulted in the cytokine gene expression in the liver but not in the tumor, spleen, and lung, whereas expression of IFN-γ was very faint, if any, in these organs (Supplementary Fig. S1). Eight hours after the transfection, serum concentration of the IL-27 p28 reached ~270 ± 2.0 pg/mL, which was significantly higher than that in the mice given a control plasmid (24.4 ± 6.1; P < 0.005, pGEG.mIL-27 versus pGEG.A). As shown in Fig. 2C, the splenic DX-5+ NK cells stimulated in vivo by IL-27 killed the target cells more vigorously in comparison with the cells that did not receive the signal.

To investigate whether IFN-γ was secreted in the sera of the tumor-bearing mice after the IL-27 gene transfection, an ELISA was performed. As shown in Fig. 2D, no IFN-γ was present in the sera of IL-27 gene–transfected mice, which is in striking contrast to the massive production of this Th1 cytokine by IL-12. Therefore, the in vivo augmentation of NK cytolytic activity showed in the IL-27 gene–transfected mice was not mediated by IL-27–induced IFN-γ.

IL-27 gene transfer significantly suppressed SCCVII tumor in mice in a NK cell–dependent fashion. To explore the effect of IL-27 on the progression of tumors in vivo, SCCVII tumor-bearing mice were repetitively transfected with IL-27 or control plasmid, and tumor growth as well as survival rates of animals were

Antibody-dependent cell-mediated cytotoxicity assay. Tumor-bearing mice or normal mice were either transfected weekly with pGEG.mIL-27 or left untreated, and sera were collected from the mice on day 28. The sera were added into wells in which effector cells and 51Cr-labeled SCCVII cells were added into wells in which effector cells and 51Cr-labeled SCCVII cells as targets were seeded at various E/T ratios (each mixture contained 25% P815 (B10.BR/2) cells were subjected to 51Cr release assay using YAC-1 (C3H/HeJ) cells as targets. Points, mean (n = 3 mice in each group); bars, SD.

Statistical evaluation. Student’s t test was used for the cytotoxicity assays, ELISA, and comparison of tumor volumes. Survival curves were computed with the Kaplan-Meier method and differences in survival rates were validated by the log-rank test. Differences were considered statistically significant at a P value of <0.05.

Results

IL-27 triggers signal transduction in NK cells. Villarino and colleagues (22) reported that IL-27RA was expressed on ~57% of NK cells obtained from C3H/HeN mice spleen. In our experiments, flowcytometric analyses showed that >95% of splenic DX-5+ cells obtained from C3H/HeN mice expressed WSX-1 as well as gp130 on

Figure 4. SCCVII cells are resistant to NK cytotoxicity, although the NK cells have been stimulated by IL-27. Splenic DX5+ cells obtained from tumor-bearing mice were cultured with 100 ng/mL of rmIL-27 or rmIL-2. Eight hours later, cells were subjected to 51Cr release assay using YAC-1 (A), SCCVII (B), and P815 (C) cells as targets. Points, mean (n = 3 mice in each group); bars, SD.
determined and evaluated. As shown in Fig. 3A, the IL-27 gene transfection led to a significant growth retardation of the preestablished tumor, in sharp contrast to the continuous and vigorous outgrowth of the control tumors. Kaplan-Meyer analysis showed that >83% of IL-27–treated mice were still alive on day 80, whereas in the same time period, only 17% of the mice given the control plasmid survived (Fig. 3B). The results strongly suggest that IL-27 induces antitumor therapeutic immunity in vivo against murine HNSCC, although IL-27 gene transfer did not lead to complete remission of the tumors (Supplementary Fig. S2).

To assess the degree of NK cell contribution to the tumor suppression activity of IL-27, tumor-bearing mice were given anti-asialo GM1 antibody to remove NK cells, and IL-27 gene transfer was performed as above. The antibody treatment considerably eliminated DX5+ NK cells, without significantly affecting CD11b+ macrophages (Supplementary Table S1). As a result, the administration of the cytokine gene was incapable of eliciting a significant antitumor outcome in the mice deprived of NK cells because their tumors showed comparable growth curves to those in the control vector-transfected group (Fig. 3C). Furthermore, NK cell depletion abrogated the survival benefit that was otherwise achieved by IL-27 gene therapy (Fig. 3D). These results clearly indicate that NK cells play indispensable roles in the antitumor effects of IL-27.

**IL-27–stimulated NK cells killed SCCVII cells via an antibody-dependent mechanism.** The experimental findings that IL-27 activates NK cells (Fig. 2) and IL-27 initiated effects suppressed SCCVII in an NK cell–dependent fashion (Fig. 3C and D) strongly suggest that NK cells effectively killed SCCVII cells after stimulation by IL-27. To assess this hypothesis, we investigated whether IL-27–provoked NK cells were actually capable of lysing SCCVII cells. Unexpectedly, however, NK cells failed to show any significant cytotoxic activity against SCCVII in vitro (Fig. 4B), despite that the effector cells had been preincubated with IL-27 or IL-2 that considerably augmented killing activity of NK cells against YAC-1 cells (Fig. 4A). Therefore, it was strongly suggested that this cancerous cell line was extremely unsusceptible to NK cytotoxicity, as were the typical NK-resistant P815 cells (Fig. 4C).

These seemingly paradoxical results can be rationalized by hypothesizing that NK cells recognized the SCCVII cells with the aid of a specific antibody and killed the target cells via an antibody-dependent cell-mediated cytotoxicity (ADCC)-based mechanism. We therefore examined whether a tumor-specific antibody was generated in the sera of SCCVII tumor–bearing mice after IL-27 gene transfection, and performed a series of cytotoxicity assays in which the sera were supplemented to the coculture of the effector and target cells. Flowcytometric analyses revealed a low level of IgG antibodies that bind SCCVII cell surface antigens in the sera of

![Figure 5](https://example.com/figure5.png)
mice bearing the tumor burden (Fig. 5B). Transfection with a control plasmid (pPEG.4) did not increase SCCVII-specific antibody in the sera of the tumor bearer (Fig. 5D), whereas IL-27 gene administration remarkably elevated the serum titer of the SCCVII-specific antibody (Fig. 5F). The genetic administration of IL-27 did not induce IgG antibody that recognized C2C12 cells, a C3H/He-derived myoblast cell line (Fig. 5C, E, and G), confirming specificity of the antibody that were induced by IL-27 gene transfer. Intriguingly, the $^{51}$Cr release assay showed that NK cells that had not been cultured with rmIL-27 were incapable of killing SCCVII cells regardless of the addition of the mouse serum (Fig. 6A), whereas IL-27–stimulated NK cells in the presence of serum successfully lysed a significant percentage of the HNSCC cells (Fig. 6B, ■). In sharp contrast, the sera collected from IL-27 gene–untransfected tumor-free mice (Fig. 6B, ▲), IL-27 gene–transfected tumor-free mice (Fig. 6B, ◆), as well as IL-27 gene–untransfected tumor-bearing mice (Fig. 6B, ♦) failed to enhance NK-mediated killing of SCCVII cells, although the effectors were prestimulated with IL-27. These results strongly suggest that NK cells activated by IL-27 killed this NK-resistant HNSCC cells in cooperation with the tumor-specific antibody(ies) induced in vivo by IL-27.

We further assessed the potential involvement of FcγRIIa (CD16) molecule in the NK-mediated lysis of SCCVII. As shown in Fig. 6C, an addition of a neutralizing CD16 antibody, but not control antibody, significantly impaired the tumoricidal activity, strongly suggesting the essential and specific contribution of the IgG-FcγRIIa interaction in HNSCC cell killing by NK cells. The suppressive effect of CD16 antibody was not complete, which leaves open a possibility that some CD16-independent mechanism(s) could also contribute to the killing of SCCVII cells by IL-27–stimulated NK cells.

These results strongly indicate that IL-27 induced tumor-specific IgG while activating NK cells, so that the antibody and the activated NK cells cooperated to kill the NK-resistant SCC cells in vivo through the ADCC machinery.

Discussion

The present study showed that IL-27 directly acted on murine NK cells to enhance their cytotoxic activity both in vitro and in vivo, while the survival rate in vitro of NK cells was also increased by this cytokine. It was also shown that therapeutic administration of the IL-27 gene drastically increased NK susceptibility of the SCCVII tumor probably through enhancing the ADCC machinery.

Cumulative evidences indicate that a pronounced function in vitro of IL-27 is to skew the differentiation of naïve CD4$^+$ T cells toward the Th1 phenotype. However, the in vivo activity of IL-27 may be to suppress immune responses as suggested by several studies using mice genetically deficient for the IL-27RA or EBI3 genes (23). Although the IL-27RA KO mice showed impaired Th1 responses at the early phase of a Leishmania major infection, the parasite was suppressed due to a significant IFN-γ production at later periods of the infection (24). The absence of the IL-27RA gene potentiated, rather than hampered, the induction of a Th1 response after infection with some intracellular pathogens including Mycobacterium tuberculosis and Trypanosoma cruzi (15, 25–28). These discrepancies between the in vitro and in vivo actions of IL-27 on T cells strongly suggest that regulatory roles of IL-27 on NK cells should be analyzed in in vivo experiments to estimate the potential usefulness of the cytokine for cancer immunotherapy. The present study indicated that IL-27 activated NK cells not only in vitro but also in vivo (Fig. 24 and C), and NK cells had an indispensable role in IL-27–mediated suppression of an HNSCC tumor (Fig. 3C and D). No significant decrease in NK cell population was revealed in the IL-27RA and EBI3 null mice (24, 29, 30). It is therefore conceivable that the IL-27 signal may not be a prerequisite for the development of NK cells, whereas supplementation of the IL-27 signal is capable of enhancing their tumoricidal and ADCC activities both in vitro and in vivo.
IL-27 may suppress tumor growth through inducing various immune responses depending on the tumor. In previous studies, mouse C26 colon carcinoma (11, 31), TBJ neuroblastoma (12), and B16F10 melanoma (32, 33) were engineered *ex vivo* to produce IL-27, and the resultant transfectants were transplanted into mice, showing significantly slower growth rates compared with their parental wild-type tumors. In some of those reports, tumor-specific CTL were induced by the cytokine that then played crucial roles in the inhibition of the IL-27–producing tumors (11, 12, 31), whereas in another report, the tumor was suppressed by IL-27–mediated induction of antiangiogenic chemokines and subsequent inhibition of neoangiogenesis (32). Furthermore, the *in vivo* deprivation of NK cells significantly restored the growth rate of the IL-27–producing tumor (33), although it remained unclear whether IL-27 activates NK cells directly or indirectly through inducing IFN-γ. Meanwhile, none of these report showed that therapeutic administration *in vitro* of IL-27 or the IL-27 gene showed an inhibitory effect against any tumor that had been preestablished in animals by transplanting wild-type neoplastic cells.

ADCC is mediated by FcγRIIa-positive NK cells in cooperation with an IgG antibody that recognizes a surface antigen on the target cells. Several cytokines including IL-2 (34–37), IL-12 (38), IL-15 (39), and IL-21 (40) augment the tumoricidal activity *in vitro* of NK cells in the presence of tumor-specific monoclonal antibodies such as rituximab (36, 37, 39), trastuzumab (40), and herceptin (35). *In vivo* studies also showed that tumors were suppressed in mice after receiving IL-2 (37), IL-12 (38), or IL-21 (40) in combination with a tumor-specific monoclonal antibody. Phase I clinical trials suggested a clinical benefit of IL-2 plus rituximab combination therapy against B-cell non–Hodgkin's lymphomas (41, 42). IL-27 may also be combined with these tumor-specific monoclonal antibodies to obtain high ADCC activity against tumor cells that are recognized by the monoclonal antibodies. Moreover, we showed that IL-27 not only enhanced NK cytotoxic activity (Fig. 2A and C) but also induced tumor-specific IgG in tumor-bearing animals (Fig. 5F). As a consequence, the growth of the NK-un susceptible tumor was remarkably suppressed without any addition of an exogenous antibody (Fig. 3). Reportedly, B cells express a functional IL-27R complex depending on the mode of activation, and IL-27 increases proliferation of anti-immunoglobulin–activated naive B cells and of anti-CD40-activated naive and germinal center B cells (13). Moreover, lipopolysaccharide plus anti-CD40-stimulated B cells were provoked by IL-27 to undergo IgG2a class switching in a STAT-1 and T-bet–dependent manner (14). IL-27RA gene–deficient mice showed a significant reduction in serum level of IgG2a (29, 43, 44). By virtue of its ability to induce both NK activation and IgG production, IL-27 may be a feasible cytokine to induce therapeutic ADCC to attack even such tumors for which appropriate monoclonal antibody have not been practically available.

IL-12 and IL-27 are homologous cytokines with Th1-inducing activities *in vitro*, and previous papers reported that IL-12 gene therapy successfully induced specific and nonspecific immune responses against SCCVII tumor in mice (45, 46). However, these cytokines are quite different from each other in terms of their capability of inducing IFN-γ *in vivo*. Omiki and colleagues (33) showed that the serum concentration of IFN-γ was remarkably elevated in IL-12–treated, but not in IL-27–treated, mice. Consistent with this is our present finding that IL-27 gene administration failed to increase the serum titer of IFN-γ, which was in striking contrast to the massive production of this proinflammatory cytokine by the IL-12 gene (Fig. 2D). In other reports, it was documented that IFN-γ was locally produced in the spleen and tumor in mice that had been transplanted with CT-26 and TBJ tumors genetically modified to secrete IL-27 (11, 12). Considering the clinical use of the cytokines for cancer therapy, the difference may be of great significance because the life-threatening toxic events associated with IL-12 immunotherapy may be mainly due to the robust production of IFN-γ (47–49). In this regard, IL-27 treatment may be more tolerated than IL-12 immunotherapy by cancer patients. Indeed, mice transplanted with IL-27–producing tumor cells did not show significant splenomegaly or liver injury that are the typical adverse effects of IL-12 (11).

The benefits and risks of IL-27 immunotherapy should also be assessed in comparison with those of IL-2, IFN-α, and IL-21 therapies that were shown to effectively suppress HNSCC in mice (20, 50). Meanwhile, IL-27 immunotherapy may be combined with other cytokine regimens that induce CTL or innate immune responses against cancer because ADCC induction is a characteristic feature of IL-27.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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