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Review Article

Development of a Whole-Cell Cancer Vaccine Containing Accumulated Intracellular Interleukin-15: Current Knowledge and Progression

Daniel P Griffin[1] and W Robert Fleischmann Jr[1]*

[1]*Foundational Sciences Discipline, Central Michigan University College of Medicine, USA*

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***Corresponding author**

W Robert Fleischmann, Foundational Sciences Discipline, Central Michigan University College of Medicine, USA, Tel: 989-774-7857; Fax: 989-774-1215; Email: fleis1wr@cmich.edu

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Abstract

Interest has focused on using potent immunostimulatory cytokines, such as IL-15, as an adjuvant for cancer treatment or as part of a vaccine therapy. This review presents an IFN-α-induced, whole-cell cancer vaccine in mice, contrasts this cancer vaccine with those reported in other studies, and considers its potential use as a human cancer vaccine. Initial studies focused on developing a B16 melanoma vaccine. B16 cells treated for ≥2 weeks with IFN-α become B16α vaccine cells that contain accumulated intracellular IL-15. Intraperitoneal, subcutaneous, and intravenous inoculations of irradiated B16α cells into mice have established adaptive immunity to B16 melanoma and the survival of a substantial fraction of the mice (60% survival with 4 vaccinations and >80% survival with 6 vaccinations). The immunity is specific to B16 melanoma; is active systemically against metastases; demonstrates memory; and, is dependent on the function of macrophages, NK cells, CD4+ helper T cells, and CD8+ cytotoxic T cells, by using corresponding knock-out mice. Thus, B16α cells are "bags" of IL-15 that express melanoma surface antigens. After inoculating irradiated B16α cells into mice, melanoma-specific tissue-infiltrating lymphocytes gather at the inoculation site. When the irradiated B16α cells lyse, they release their accumulated IL-15 as a bolus, activating the melanoma-specific tissue-infiltrating lymphocytes. The activated lymphocytes proliferate and kill B16 melanoma cells throughout the body. While initial studies were focused on developing B16α cells as a melanoma vaccine, the IFN-α treatment protocol has been employed to develop RM-1α cells and P388α cells as vaccines against RM-1 prostate cancer and against P388 lymphocytic leukemia, respectively. The demonstration of efficacious vaccines against multiple cancers supports the general applicability of the IL-15-containing whole-cell vaccine. The relative efficacies of the different vaccines appear to be associated with a relative down-regulation of translation of the IL-15 mRNA. To achieve the full potential of the vaccines, it will be necessary to transfect cancer cells with constructs of IL-15 that negate the down-regulatory mechanisms. Since mouse and human immune systems illustrate many common features of IL-15 function, these studies have high promise of being extended to the creation of human cancer vaccines.

Introduction

It is estimated that more than 1.68 million people in the United States of America will develop cancer in 2016 [1]. Most of the current treatments can be very deleterious to patients: surgeries can be disfiguring and radiation and chemotherapies can be associated with significant toxicities. Despite these therapies, more than a third of these individuals, an estimated 595 thousand people in 2016, will succumb to the disease [1]. Moreover, many of those who do survive will have to deal with disfigurement; heart and kidney damage; memory problems; intestinal problems; incontinence and impotence; and, the induction of other cancers [2]. Cancer is a similar scourge in other countries. Thus, there continues to be a desperate need for new therapies that exhibit greater efficacies and lower toxicities.

Cancer Immunosurveillance

Our cells constantly accumulate mutations. The mutations occur because of the effects of the physical (radiation) and chemical environment (environmental contaminants); chemical resonance of our DNA bases; and, biological limitations of our replication machinery. Repair systems correct many of these mutations but some mutations become permanent as part of our cells' genetic makeup.

Cancer cells arise when permanent mutations occur in proto-oncogenes and tumor suppressor genes. Fortunately, the body has an immunosurveillance system that recognizes and eliminates isolated precancerous and cancerous cells as they arise [reviewed in 3-4]. The immunosurveillance system is highly efficient but not without its fault. Thus, if a cancer cell is not recognized and killed by the immunosurveillance system, it may eventually begin to divide and form a collection of cancer cells. There are a number of mechanisms by which cancer cells escape immunosurveillance. In one of these mechanisms, even though the host immune system can still recognize the collection of cancer cells, the immune system's lymphocytes become tolerant of these cancer cells and do not kill them [4]. This development of tolerance can lead to the growth of the cancer cells to sufficient

size to be detected, to cause physical problems, and to be diagnosed as "cancer."

Rationale for Immunotherapy

Even when tolerance to cancer cells has developed, T cells of the immune system continue to localize in the tumor mass. Such T cells are the major component of the tumor-infiltrating lymphocytes [5-7]. The tumor-infiltrating lymphocytes are tolerant to the cancer cells, as they still recognize the cancer cells but can no longer kill them. The potential power of tumor-infiltrating lymphocytes to kill foreign cells can be seen with their potent activity in the rejection of tissue and organ transplants.

If these tolerant tumor-infiltrating lymphocytes could be re-activated to break their tolerance, they would exert a potent cancer-killing effect. Studies have suggested that tolerance can be broken. For example, *in vitro* treatment of tolerant tumor-infiltrating lymphocytes with a potent immunostimulatory molecule (IL-2) has been shown to be able to re-activate the previously tolerant tumor-infiltrating lymphocytes to attack the cancer cells [8]. These studies were extended to human clinical trials [9]. When the *in vitro* IL-2-treated tumor-infiltrating lymphocytes were injected into melanoma patients, they killed the melanoma cells, resulting in complete tumor regressions in 22% of patients, allowing an extended survival of the patients [9]. It should be noted that, to achieve these complete responses, the patients had to first be treated with drugs to deplete their lymphocytes [9]. Even so, the results suggest that immunotherapies targeted toward re-awakening the patient's own immune system have great promise.

A number of additional immunotherapy approaches to re-activate the immune system are currently under investigation, including vaccination with genetically modified cancer cells; cancer-specific antigens; dendritic cells and T cells reactivated *in vitro* by exposure to cancer antigens and/or cytokines; genetically modified viruses and other vectors; and, antibodies to various immune cell surface markers [for recent overview, see 10]. There are strengths and weaknesses to each of these approaches.

This review focuses on our laboratory investigations into the development of IL-15-based, whole cell cancer vaccines to re-activate the host immune system.

Results

Discovery of a Melanoma Cancer Vaccine

In a mouse cancer model, it has long been known that B16 melanoma cells are very poorly immunogenic or non-immunogenic and, thus, are seen poorly by the mouse immune system. This has made B16 melanoma an excellent model for studying the antitumor effect of IFN-α. Unfortunately, while IFN-α therapy has initial antitumor activity, resistance to interferon therapy quickly develops.

While studying the development of resistance to the antitumor effect of interferon, a serendipitous discovery was made [11]. Namely, B16 melanoma cells that had been treated for two weeks or more with IFN-α (B16α cells) could be effectively seen by the immune system and could stimulate a potent antitumor activity.

Acting on this observation, B16α cells were tested for their ability to act as a vaccine against the parental, untreated B16 melanoma cells [12]. B16α cells were exposed to concentrations of IFN-α ranging from 300 to 10,000 international units/ml. Following two weeks of treatment, the treated B16α cells were lethally irradiated and inoculated four times, at weekly intervals, into mice. The mice were then challenged with inoculations of live, untreated B16 melanoma cells. In Figure 1, it can be seen that none of the challenged mice survived when inoculated four times with carrier. However, mice inoculated four times with irradiated B16α cells that had been cultured in various concentrations of IFN-α survived for longer periods of time. Further, some cures were noted. Moreover, the efficacy of treatment with B16α cells was dependent upon the dose of IFN-α to which the B16α cells had been exposed and seemed to be defined as a bell-shaped dose-response curve. B16α cells treated with 3,000 units of IFN-α provided the significantly greatest protective effect, with 60% of the mice surviving live B16 melanoma cell challenge. Treatment of B16α cells with greater or less than 3,000 units of IFN-α provided a lower protective effect. The underlying mechanism for the significantly decreased efficacy of the cancer vaccine to IFN-α treatment levels above 3,000 units of IFN-α is unknown, but it is suspected to be due to the stimulation of a down-regulatory mechanism.

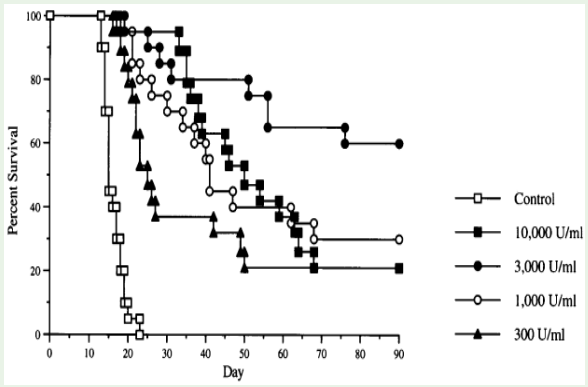

Figure 1: Effect of *in vitro* IFN-α treatment concentration. Mice (20 mice/group) were inoculated i.p. on days -21, -14, -7, and 0 with 10^6 inactivated B16α cells. The inactivated B16α cells were pretreated for more than 14 days with 300, 1000, 3000, or 10,000 international reference units of IFN-α. Control mice were mock inoculated with the carrier (Hanks Basal Salt Solution). On day 1, all mice were challenged i.p. with 10^6 live B16 cells. The graph plots the combined data of two experiments as cumulative survival vs. day after tumor inoculation. Day of death was noted. Statistical analysis by Fisher exact probability test: 3,000 U/ml versus 300 U/ml: p = 0.0016; 3,000 U/ml versus 1,000 U/ml: p = 0.043; 3,000 U/ml versus 10,000 U/ml: p = 0.021. Reprinted from reference 12. Used with the permission of Mary Ann Liebert, Inc.

Further studies showed that the vaccine efficacy of the irradiated B16α cells increased with increasing numbers of inoculations [13]. Four inoculations with irradiated B16α cells protected 50% of the mice from challenge with live B16 melanoma. However, six vaccinations protected more than 80% of the mice from challenge.

The results of these experiments strongly indicate that the irradiated B16α cells provide a highly significant protective effect in mice challenged with B16 melanoma.

Vaccine Induces Adaptive Immunity

The results of these experiments strongly suggest that the irradiated B16α cells are behaving as a vaccine for B16 melanoma. A true vaccine induces adaptive immunity. Therefore, to claim that

Table 1: Potency of Vaccination with Inactivated B16 or B16α Cells against B16-F10 Lung Metastases.

Vaccination Cell Type[a]	Number of Mice Evaluated	Lung Metastases Mean ± SE Lung	% Decrease in Lung Metastases[b] ± SE
1. None	20	150.8 ± 8.8	
2. B16	19	145.6 ± 9.5	3 ± 6
3. B16α	20	52.5 ± 5.8	65 ± 4

[a]C57BL/6 mice were vaccinated with 10^6 inactivated B16 or B16α cells on days -21, -14, -7, and 0 before challenge with 5 x 10^5 live B16-F10 cells via tail vein on day 0. Mice were killed on day 16 for evaluation of lung metastases. Control mice received only mock vaccinations with carrier (Hanks Basal Salt Solution).
[b]Student's *t*-test analysis of % decrease in lung metastases: group 1 versus group 2: p = NS; group 1 versus group 3: p < 0.0001; group 2 versus group 3: p < 0.0001.NS: not significant.
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the B16α vaccine is a true vaccine, it was necessary to prove that the protective effect was the result of induction of adaptive immunity and not due to the induction of innate immunity.

First, the ability of the B16α cells to establish a systemic effect was determined. Irradiated B16α cells were inoculated intraperitoneally [12]. The vaccinated mice were then challenged by tail vein injection with the F10 clone of B16 melanoma. The F10 clone preferentially metastasizes to the lung. The results of Table 1 show that inoculation with irradiated B16 cells that have been grown in the absence of interferon do not provide a protective effect. However, inoculation with irradiated B16α cells reduces the number of lung metastases by 65%.

Second, the specificity of the antitumor effect induced by B16α cells was determined [14]. Irradiated B16α cells were inoculated into mice that were then challenged with live B16 melanoma cells or with live RM-1 prostate cancer cells. B16α vaccination protected 50% of mice challenged with live B16 melanoma cells, but 0% of mice challenged with live RM-1 prostate cancer cells.

Third, the ability of B16α cell inoculation to establish memory was determined [12]. Mice were inoculated with irradiated B16α cells and challenged with live B16 cells. After 90 days, the surviving mice were then re-challenged with live B16 cells. The results of Table 2 show that

Table 2: Resistance of Surviving Vaccinated Mice to B16 Re-challenge.

Vaccination Group[a]	Booster	Number of Survivors of First Challenge	Number of Survivors of Second Challenge[b]
1.None[c]	None	0/20	
2.None[d]	None	0/10	
3.B16α	None	10/20	3/10
4.B16α	One: B16α	13/18	12/13

[a]C57BL/6 mice were vaccinated with 10^6 inactivated B16α cells on days -21, -14, -7, and 0 before i.p. challenge with 10^6 live B16 cells on day 0.The survivors of the initial challenge were given either one booster vaccination consisting of 10^6 inactivated B16α or one mock vaccination (carrier) on day 90, re-challenged with 10^6 live B16 cells on day 93, and monitored for their survival for another 90 days. Control mice received only mock vaccinations with carrier (Hanks Basal Salt Solution).
[b]Fisher exact probability test for the number of re-challenge survivors: group 2 versus group 3: NS; group 2 versus group 4: p <0.0001; group 3 versus group 4: p <0.0032.NS: not significant.
[c]Control mice for the first challenge (challenged on day 0).
[d]Control mice for the second challenge (challenged on day 93).
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thirty percent of the mice survived the re-challenge, indicating that the inoculation with B16α cells established an enduring antitumor effect. Most interestingly, when mice were given a single booster inoculation with B16α cells, prior to re-challenge with live B16 cells, more than 90% of the mice survived the re-challenge.

Fourth, the immune cells responsible for mediating the antitumor effect induced by inoculation with B16α cells were determined [15]. Knockout mice that we depleted of IL-12, Natural Killer (NK) cells, CD8+ cytotoxic T cells, CD4+ helper T cells, or B cells were inoculated with irradiated B16α cells and challenged with live B16 melanoma cells (Table 3). IL-12 knockout mice had only 20% protection as compared with 47% for normal mice. NK cell knockout mice had only 20% protection as compared with 50% for normal mice. CD8+cytotoxic T cell knockout mice had only 15% protection as compared with 48% for normal mice. Finally, CD4+helper T cell knockout mice had 0% protection, as compared with 50% for normal mice, suggesting that there was no role for innate immunity in the survival of the B16α-vaccinated mice. Knockout mice that were depleted of B cells did not show a reduced level of protection, indicating that B cells are not mediators of the B16α-induced antitumor activity.

The results of these experiments show that inoculation with irradiated B16α cells provides a systemic effect, is specific to the B16 melanoma, and establishes a memory response. Taken together, the

Table 3: Effect of IL-12, CD8+ T Cell, NK Cell, CD4+ T Cell, or B Cell Deletion on Vaccinated State.

Vaccination Cell Type[a]	Mouse Type	Number of Survivors	% Survivors	Statistical Significance[b]
1. None 2. B16α 3. B16α	Normal Normal IL-12 Knockout	0/27 15/32 4/20	0 47 20	Groups 1 *vs.* 2: p<0.0001 Groups 1 *vs.* 3: p = 0.027 Groups 2 *vs.* 3: p = 0.036
4. None 5. B16α 6. B16α	Normal Normal CD8 Knockout	0/20 12/25 3/20	0 48 15	Groups 4 *vs.* 5: p = 0.0004 Groups 4 *vs.* 6: p = NS Groups 5 *vs.* 6: p = 0.027
7. None 8. B16α 9. B16α	Normal Normal Beige (NK KO)	0/20 10/20 3/20	0 50 15	Groups 7 *vs.* 8: p = 0.0002 Groups 7 *vs.* 9: p = NS Groups 8 *vs.* 9: p = 0.018
10. None 11. B16α 12. B16α	Normal Normal CD4 Knockout	0/27 10/20 0/20	0 50 0	Groups 10 *vs.* 11: p <0.0002 Groups 10 *vs.* 12: p = NS Groups 11 *vs.* 12: p <0.0002
13. None 14. B16α 15. B16α	Normal Normal B cell-deficient	0/20 12/20 16/20	0 60 80	Groups 13 *vs.* 14: p <0.0001 Groups 13 *vs.* 15: p <0.0001 Groups 14 *vs.* 15: p = NS

[a]C57BL/6 and knockout mice (C57BL/6 background) were vaccinated with 10^6 inactivated B16α cells on days -21, -14, -7, and 0 prior to i.p. challenge with 10^6 live B16 cells on day 0. Control mice received only mock vaccinations with carrier (Hanks Basal Salt Solution).
[b]Fisher's exact probability test for the % mice protected. NS: not significant.
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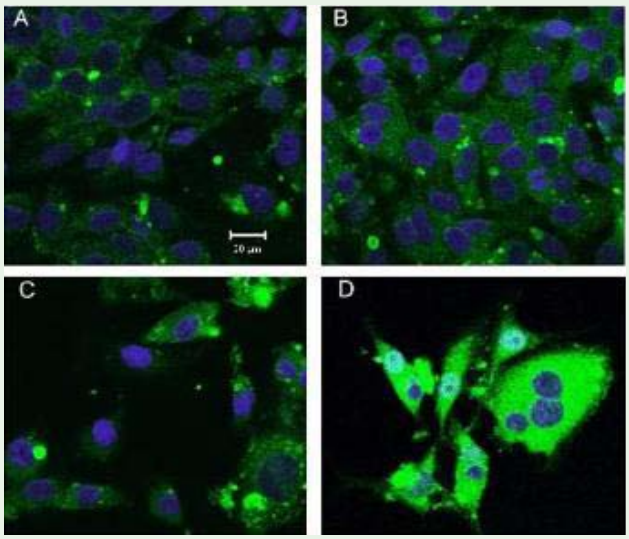

Figure 2: Subcellular distribution of IL-15 protein in B16*α* vaccine cells. B16 parental cells (**A**,**B**) and B16*α* vaccine cells (**C**,**D**) were cultured in the absence or presence of 1000 international reference units of IFN-*α*, respectively. The cells were fixed, permeabilized, and incubated with (**B**,**D**) or without (**A**,**C**) primary antibody to IL-15 and subsequently incubated with secondary antibody to IgG conjugated with FITC (green fluorescence). Nuclei were stained with DAPI (blue fluorescence). Fluorescence was visualized with a Zeiss LSM510 UV META confocal microscope. The illustrations represent overlays of the blue and green fluorescence images. All images have the same magnification, with the scale indicated in **A**. (**A**) B16 parental cells incubated without primary antibody to IL-15. (**B**) B16 parental cells incubated with primary antibody to IL-15. (**C**) B16*α* vaccine cells incubated without primary antibody to IL-15. (**D**) B16 vaccine cells incubated with primary antibody to IL-15. Reprinted from reference 16.Used with the permission of Mary Ann Liebert, Inc.

results indicate that the irradiated B16*α* cells establish a true adaptive immune response that is mediated by macrophages, NK cells, CD8+ cytotoxic T cells, and CD4+ helper T cells. Not unexpectedly, CD4+ helper T cells are the most important immune cells. Thus, irradiated B16*α* cells are an efficacious cancer vaccine.

B16*α* cells Contain Cell-Associated IL-15

Irradiated B16*α* cells were an efficacious vaccine, but irradiated B16 cells were not. What was different about the B16*α* cells that made them an effective vaccine? B16*α* cells did not have greater expression of melanoma antigen nor did they have greater levels of endogenous virus antigens [11]. B16*α* cells were shown not to secrete detectable levels of cytokines, including IL-15 [16].

A key experiment led to an understanding of the difference between B16*α* cells and B16 cells that could explain the vaccine status of the B16*α* cells. Immune cells from the spleens of mice inoculated with irradiated B16*α* cells or with irradiated B16 cells were placed in tissue culture in the absence of cytokine growth factors [16]. As expected, spleen cells from mice inoculated with irradiated B16 cells showed only a background level of thymidine incorporation. However, spleen cells from mice inoculated with irradiated B16*α* cells spontaneously proliferated. The spleen cells showed a 5-fold level of thymidine incorporation above background at 1 week after the last inoculation with irradiated B16*α* cells and a 4-fold level of thymidine incorporation above background at 5 weeks after the last inoculation. This observation suggested that the mouse immune cells from the spleens had been powerfully stimulated to proliferate *in vivo* and that this powerful stimulation persisted for at least 5 weeks after the last inoculation.

IL-2 and IL-15 are both potent immunostimulatory cytokines. They have many properties in common, but also have some important differences [17-22]. T cells stimulated with IL-2 are activated to proliferate and then undergo apoptosis after a relatively short period of time. T cells stimulated with IL-15 are activated to proliferate and maintain that activation for a relatively long period of time. The results above suggested that the spleen cells had been activated by IL-15 [16].

B16*α* cells and B16 cells were evaluated for IL-15 mRNA and protein expression in cell lysates. There was an 85-fold enhancement in IL-15 mRNA synthesis and 8-fold enhancement of IL-15 protein in B16*α* cells over B16 cells [16].

Since secreted IL-15 was not detected, B16*α* and B16 cells were subjected to confocal fluorescence microscopy. These studies (Figure 2) demonstrated that B16*α* cells contained an abundance of intracellular IL-15, while B16 cells had only little immunoreactive staining. Moreover, the distribution of the intracellular IL-15 in the B16*α* cells indicated that the IL-15 was not limited to the Golgi apparatus. Rather, the IL-15 was distributed throughout the cytoplasm and even in the nucleus of the B16*α* cells.

Thus, long-term IFN-*α* treatment of B16*α* cells caused the production and intracellular accumulation of IL-15.

Broad Applicability of Vaccine Protocol

Convincing evidence showed that long-term IFN-*α* treatment of B16 cells can result in the development of a potent anti-cancer B16*α* vaccine against B16 melanoma. It was important to know whether this was a specific or general phenomenon. RM-1 prostate cancer cells and P388 lymphocytic leukemia cells were treated for two weeks with IFN-*α* to create RM-1*α* cells and P388*α* cells [14]. Mice were inoculated with irradiated RM-1*α* cells or irradiated P388*α* cells and challenged with live RM-1 cells and live P388 cells, respectively. A substantial protective effect was observed for both RM-1*α* cells (20% survival with 6 vaccinations) and P388*α* cells (25% survival with 4 vaccinations) for this experiment. In each case, inoculation with irradiated RM-1 cells and irradiated P388 cells, grown in the absence of IFN-*α* did not provide any protective effect.

Limitation of Vaccine Protocol

The levels of protection were seen to be lower for RM-1*α* cells and for P388*α* cells than had been seen for B16*α* cells (an experiment-to-experiment range of 50-60% protective effect for 4 vaccinations with B16*α* versus an experiment-to-experiment range of 20-60% protective effect for 6 vaccinations with RM-1*α*). Consequently, intracellular IL-15 levels were compared for RM-1*α* cells and B16*α* cells, with and without irradiation of the vaccine cells [23]. The level of intracellular IL-15 was initially lower in RM-1*α* cells but increased approximately 3-fold with irradiation. The initially higher level of intracellular IL-15 in B16*α* cells was unaffected by irradiation. So, the irradiation data suggested that irradiation may have partially negated a possible control mechanism operating in the RM-1*α* cells, but not in the B16*α* cells, that limits IL-15 synthesis.

Discussion

There is a great deal of excitement about the potential use of IL-15 as immunotherapy [reviewed in 24-26]. Thus, a number of studies have already been undertaken.

Therapies Based on IL-15 Administration

While the results of clinical trials with IL-15 are not yet available, several preclinical studies have shown promise. Often, IL-15 has been paired with another immuno-active molecule for improved therapeutic efficacy. In one study [27], administration of IL-15 alone provided about 20% survival in mice with TRAMP-C2 prostate cancers. This level of survival could be increased to more than 60% when IL-15 therapy was combined with antibodies directed against two regulatory proteins on cytotoxic T cells.

A later study [28], using a combination of IL-15 and IL-15Rα to treat mouse melanoma, suggests that the combination of IL-15 and its receptor, could be a more efficacious NK cell-mediated approach to both slow tumor progression and reduce tumor volume.

Therapies Based on Expression Vectors

An intra-tumoral injection of adenovirus expressing the IL-15 gene paired with the chemokine gene, CCL21, in colon carcinoma challenged mice [29] resulted in greater reduction of tumor growth compared to the effects of administration of either IL-15 or CCL21, independently.

In Vivo IL-15 Treatment of Immune Cells

In mice bearing colon carcinoma tumors, combination treatment with IL-15 injection (in liposomes) and whole cell vaccine reduced tumor growth by 45% compared with tumor growth occurring in mice treated with IL-15 alone or with whole cell vaccine alone [30].

Mice bearing Lewis lung carcinoma, when treated with liposomes carrying IL-15 gene-inserted-plasmid co-administered with an autologous whole-cell tumor vaccine protected mice from challenge, resulting in significantly reduced tumor volume and increased median survival percentage than those resulting from independent treatment with either the liposomes carrying the IL-15 gene or whole-cell tumor vaccine [31].

In Vitro IL-15 Treatment of Immune Cells

In studies on *in vitro* IL-15 activation of T cells, *in vitro* treatment with IL-15 caused 10^5-10^6-fold proliferation of tumor antigen-specific memory T cells and prevented early apoptosis of the T cells [32]. Also, IL-15 treatment inhibited Treg expansion.

In addition to studying the effect of IL-15 on activation of T cells, recent attention has focused on dendritic cells. Dendritic cell vaccinations supplemented with co-expression of IL-15 and its receptor, IL-15Rα, have been shown to enhance the immune response. Treatment of rat neu-expressing transgenic mice with dendritic cells co-expressing a truncated form of the rat neu gene plus mouse IL-15 and IL-15Rα, protected approximately 70% of mice from mammary carcinomas at 30 weeks. In contrast, there was 0% protection of these transgenic mice treated with dendritic cells expressing only the truncated neu gene, 20% protection by dendritic cells expressing the truncated neu gene and IL-15Rα, and only 10% protection by dendritic cells expressing the truncated neu gene and IL-15 [33].

The use of dendritic cell-based cancer vaccines has mostly focused on enhancing their antigen presenting properties, thereby more effectively stimulating antigen-specific cytotoxic T cells. But, more recently, it has been demonstrated that *in vitro* treatment with IL-15 activates peripheral blood monocytes to become dendritic cells, known as IL-15 DCs. This activation results in the cell surface expression of IL-15 on the dendritic cells, and the subsequent activation of NK cells by contact [34, reviewed in 35]. Another study carried this concept further by employing dendritic cells that were transfected with both IL-15 and IL-15 receptor to activate NK cells [36]. These studies demonstrate the power of IL-15, particularly together with IL-15Rα, to activate the host immune system.

Administration of IL-15-Producing Cells

Cancer cells transfected with the secretable IL-15 gene construct has been monitored for their ability to establish immunity in mice. Studies have involved IL-15-transfected prostate cancer cells [37], colon carcinoma cells [38], and fibrosarcoma cells [39]. Dramatic effects, including cures, were observed with these IL-15-transfected cells after they were inoculated into immunocompetent and even into immunocompromised mice. However, the IL-15-transfected cells were not lethally irradiated prior to inoculation, as they would have to be for human studies.

In mice, cells transfected with expression vectors containing the gene for codon-optimized IL-15 have been developed [40]. Codon-optimization gave a 100-fold greater secretion of IL-15 by cells transfected with the expression vector *in vitro*. After *in vivo* inoculation, detectable levels of circulating IL-15 were observed, as were increased numbers of NK cells in the liver, spleen, and lung. The effect of this therapy on tumors was not evaluated. This study shows the power of codon-optimization for increasing IL-15 translation. However, it also raises concerns for side effects of systemically distributed IL-15, such as the potential for autoimmune disease.

Cancer cells expressing both IL-15 and its receptor have also been a more recent approach. Co-expression of IL-15 and its receptor, IL-15Rα, in mouse breast and prostate cancer cells increases the cell-surface expression and secretion of IL-15 above that found in cells expressing IL-15 alone. Vaccinations of mice with these co-expressing cells produced CD8+cytotoxic T cell-mediated and NK cell-mediated anti-tumor effects, including the inhibition of tumor growth at sites distant from the initial site of inoculation [41,42]. These transfected cells were treated with mitomycin C to inhibit replication (and transcription). Thus, while the studies show the power of IL-15, they may not predict success in humans because the mitomycin C-treated vaccine cells may not continue to secrete IL-15.

Limitations of These Studies. Preclinical studies show that IL-15 has potent effects in both mice and humans [reviewed in 24-26]. However, it can be anticipated that there will be problems with ultimate clinical administration of IL-15 or IL-15 expression vectors, as significant side effects have already been noted for IL-15 treatment in human trials, requiring the treatment to be limited to, at most, every third day [43]. Also, vaccine therapies based upon secreted IL-15-producing cells may not succeed in clinical trials if lethal irradiation is employed because lethal irradiation may compromise the production of the secreted IL-15. The most promising preclinical study would seem to be one that would involve the re-inoculation of harvested immune cells that have been treated *in vitro* with IL-15 to activate the

immune cells. Potential concerns with ultimate clinical application of this approach would be that there may be possible limitations on the number of immune cells that can be harvested and that there may be possible limitations on the effectiveness of the *in vitro* treatment.

IFN-α Induced Vaccine Contains IL-15. The studies reported herein demonstrate that a highly efficacious whole-cell cancer vaccine can be generated in mice by long-term treatment of the cancer cells with IFN-α. The B16α vaccine is a true vaccine that induces an adaptive immune response that is characterized by its ability to establish a systemic effect, to be specific against a particular cancer type (B16 melanoma), and to establish memory T cells. Macrophages, natural killer cells, CD8+ cytotoxic T cells, and CD4+ helper T cells all play important roles in mediating the potent effects of the B16α cancer vaccine. Studies investigating the mechanism by which the B16α vaccine induces its activation of the immune system have shown that long-term IFN-α treatment induces the production of cell-associated IL-15 in the B16α vaccine cells.

In the literature, there is support for IL-15's role in stimulating the immune system to attack cancer cells. For example, IL-15 has been shown to have a role in immunosurveillance in a mammary carcinogenesis study comparing IL-15 wild-type and IL-15 knockout HER2/neu transgenic mice. The IL-15 knockout mice had earlier mammary carcinogenesis and had significantly reduced levels of NK and CD8+ cytotoxic T cells [44].

Uniqueness of an IL-15-Based Vaccine

The essential feature of the vaccine described herein is that the IFN-α treatment induces cell-associated IL-15. In this regard, two isoforms of IL-15 have been described: one IL-15 isoform is secreted, while the other IL-15 isoform accumulates intracellularly [45-47]. The IL-15 isoform that is induced in the B16α vaccine cells described herein is the isoform that accumulates intracellularly.

Other studies have shown that IFN-α treatment induces IL-15 in dendritic cells [48] and in hepatocellular carcinoma cells [49]. However, in these studies, the type of IL-15 isoform that was produced was not identified and the effect of IL-15 on the induction of antitumor activity was not addressed.

The other cited studies employ vaccine cells that secrete IL-15 (24-42). Thus, the B16α vaccine with its accumulated cell-associated IL-15 is unique.

How is the B16α vaccine envisioned to generate its immunostimulatory effect? The B16α vaccine cells are essentially "bags" of IL-15 that express B16 cancer antigens on their surface. After lethal irradiation and inoculation into the host, tissue-infiltrating lymphocytes that recognize, but do not respond to the expressed B16 cancer antigens, accumulate at the site of the inoculation. After 2-3 days, the irradiated B16α cancer vaccine cells lyse, releasing their accumulated intracellular IL-15 as a bolus in the local area of the tissue-infiltrating lymphocytes that specifically recognize the B16 cancer antigens. The IL-15 then activates the B16-specific tissue-infiltrating lymphocytes (primarily Th1, Th2, and NK cells) to overcome their tolerance, to begin to proliferate. Thus, when the mice are challenged with live B16 cancer cells, the activated tissue-infiltrating lymphocytes recognize the live B16 cancer cells and kill them, permitting the survival of the B16α vaccinated mice.

Universality of Vaccine Protocol

The protocol used to develop B16α vaccine cells can be used to develop vaccine cells for RM-1 prostate cancer and for P388 lymphocytic leukemia in mice. It should be noted that the RM-1α vaccine, as well as the B16α melanoma vaccine protect against solid tumors, while the P388α vaccine protects against a leukemic malignancy. So, the vaccine protocol has shown promise against solid and against hematological malignancies. Also, the RM-1α and B16α vaccines are syngeneic to the C57BL/6 mouse, while the P388α vaccine is syngeneic to the DBA/2 mouse. So, the vaccine protocol is not limited to a given mouse strain and has promise in mice with different major histocompatibility antigens.

These studies have proven the principle in the mouse system that highly efficacious cancer vaccines can be created by the induction of IL-15 in cancer cells by long-term IFN-α treatment. Moving forward, the best method to create cancer vaccines will most probably involve the direct transfection of cancer cells with the IL-15 gene. Studies with RM-1α prostate cancer vaccine cells strongly suggest that IL-15 mRNA translation is down-regulated. Necessarily, future studies will be focused on the transfection of cancer cells with IL-15 constructs that avoid the down-regulation of IL-15 translation that appears to limit IL-15 induction in some cancer cells.

The IL-15-based, whole-cell vaccine has several advantages. First, the vaccine combines the potent immunostimulatory cytokine IL-15 and the cancer cell antigens in one "package". Second, the vaccine will attract those host T cells that recognize the cancer cell antigens and release the IL-15 locally to specifically activate those cancer antigen-specific T cells. Third, multiple vaccinations can be given to boost the activation of the host immune system response against the cancer. Fourth, the IL-15-based, whole-cell vaccine can be tailored for a patient's specific cancer, using biopsy materials.

There are great similarities between IL-15 induction and function in mouse and human systems. Thus, there is strong reason to believe that this work, developing a mouse vaccine based upon the accumulation of intracellular IL-15, will extend to the human system. Therefore, it will be important to determine whether IL-15-transfected human cancer cells that accumulate intracellular IL-15 can be efficacious vaccines. If so, successful extension of these studies to the human system should lead to the development of a protocol for the generation of highly efficacious, IL-15-based, whole-cell cancer vaccines against a number of cancers.

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References

1. http://www.cancer.org
2. http://www.mayoclinic.com/health/cancer-survivor/CA00073
3. Bui JD, Schreiber RD. Cancer immunosurveillance, immunoediting and inflammation: independent or interdependent processes? Curr Opin Immunol. 2007; 19: 203-208.
4. Dunn GP, Bruce AT, Ikeda H, Old LJ, Schreiber RD. Cancer immunoediting: from immunosurveillance to tumor escape. Nat Immunol. 2002; 3: 991-998.

5. Murray D, Hreno A, Dutton J, Hampson LG. Prognosis in colon cancer: a pathologic reassessment. Arch Surg. 1975; 110: 908-913.

6. Miescher S, Stoeck M, Qiao L, Barras C, Barrelet L, et al. Proliferative and cytolytic potentials of purified human tumor-infiltrating T lymphocytes. Impaired response to mitogen-driven stimulation despite T-cell receptor expression. Int J Cancer. 1988; 42: 659-666.

7. Schriber H. Tumor immunology. In: Fundamental immunology. Paul WE (editor). Philadelphia, PA, USA: Lippencott, Williams & Wilkins. 2003; 1557-1592.

8. Grimm EA, Mazumder A, Zhang HZ, Rosenberg SA. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. J Exp Med. 1982; 155: 1823-1841.

9. Rosenberg SA, Yang JC, Sherry RM, Kammula US, Hughes MS, Phan GQ, et al. Durable complete responses in heavily pretreated patients with metastatic melanoma using T-cell transfer immunotherapy. Clin Cancer Res. 2011; 17: 4550-4557.

10. www.cancer.org/acs/groups/cid/documents/webcontent/003013-pdf.pdf

11. Fleischmann CM, Wu TY, Fleischmann WR. B16 melanoma cells exposed in vitro to long-term IFN-alpha treatment (B16 alpha cells) as activators of tumor immunity in mice. J Interferon Cytokine Res. 1997; 17: 37-43.

12. Wu TY, Fleischmann WR. Efficacy of B16 melanoma cells exposed in vitro to long-term IFN-alpha treatment (B16alpha cells) as a tumor vaccine in mice. J Interferon Cytokine Res. 1998; 18: 829-839.

13. Fleischmann WR, Wu TG. Development of an interferon-based cancer vaccine protocol: application to several types of murine cancers. Methods Mol Med. 2005; 116: 151-166.

14. Fleischmann WR, Wu TG. Development of a vaccine against cancers. Razvoj Cepiva Proti Raku. 2004; 43: S5: 51-61.

15. Wu TY, Fleischmann WR. Murine B16 melanoma vaccination-induced tumor immunity: identification of specific immune cells and functions involved. J Interferon Cytokine Res. 2001; 21: 1117-1127.

16. Wu TG, Rose WA II, Albrecht TB, Knutson EP, Konig R, et al. IFN-alpha-induced murine B16 melanoma cancer vaccine cells: induction and accumulation of cell-associated IL-15. J Interferon Cytokine Res. 2007; 27: 13-22.

17. Bamford RN, Battiata AP, Burton JD, Sharma H, Waldmann TA. Interleukin (IL) 15/IL-T production by the adult T-cell leukemia cell line HuT-102 is associated with a human T-cell lymphotrophic virus type I region /IL-15 fusion message that lacks many upstream AUGs that normally attenuates IL-15 mRNA translation. Proc Natl Acad Sci U S A. 1996; 93: 2897-2902.

18. Berard M, Brandt K, Bulfone-Paus S, Tough DF. IL-15 promotes the survival of naive and memory phenotype CD8+ T cells. J Immunol. 2003; 170: 5018-5026.

19. Dubois S, Patel HJ, Zhang M, Waldmann TA, Muller JR. Preassociation of IL-15 with IL-15R alpha-IgG1-Fc enhances its activity on proliferation of NK and CD8+/CD44 high T cells and its antitumor action. J Immunol. 2008; 180: 2099-2106.

20. Grabstein KH, Eisenman J, Shanebeck K, Rauch C, Srinivasan S, Fung V, et al. Cloning of a T cell growth factor that interacts with the beta chain of the interleukin-2 receptor. Science. 1994; 264: 965-968.

21. Klebanoff CA, Finkelstein SE, Surman DR, Lichtman MK, Gattinoni L, Theoret MR, et al. IL-15 enhances the in vivo antitumor activity of tumor-reactive CD8+ T cells. Proc Natl Acad Sci USA. 2004; 101: 1969-1974.

22. Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. J Exp Med. 1995; 181: 1255-1259.

23. Wu TG, Perdigão JR, Umhoefer TK, Cao J, Ansari DA, Albrecht TB, Knutson EP. Heterogeneous interleukin-15 inducibilities in murine B16 melanoma and RM-1 prostate carcinoma by interferon-alpha treatment. J Interferon Cytokine Res. 2009; 29: 719-728.

24. Jakobisiak M, Golab J, Lasek W. Interleukin 15 as a promising candidate for tumor immunotherapy. Cytokine Growth Factor Rev. 2011; 22: 99-108.

25. Croce M, Orengo AM, Azzarone B, Ferrini S. Immunotherapeutic applications of IL-15. Immunotherapy. 2012; 4: 957-969.

26. Ochoa MC, Mazzolini G, Hervas-Stubbs S, de Sanmamed MF, Berraondo P, Melero I. Interleukin-15 in gene therapy of cancer. Curr Gene Ther. 2013; 13: 15-30.

27. Yu P, Steel JC, Zhang M, Morris JC, Waitz R, et al. Simultaneous inhibition of two regulatory T-cell subsets enhanced interleukin-15 efficacy in a prostate tumor model. Proc Natl Acad Sci USA. 2012; 109: 6187-6192.

28. Isvoranu G, Marinescu B, Surcel M, Ursaciuc C, Manda G. Immunotherapy in Cancer - In vivo study of the anti-tumor activity of the IL-15/IL-15R alfa combination in an experimental model of melanoma. Farmacia. 2015; 63: 631-636.

29. Zhao DX, Li ZJ, Zhang Y, Zhang XN, Zhao KC, Li YG, et al. Enhanced antitumor immunity is elicited by adenovirus-mediated gene transfer of CCL21 and IL-15 in murine colon carcinomas. Cell Immunol. 2014; 289: 155-161.

30. Zhao YW, Li ZM, Zhao CJ, Zhang L, Jin L, Wang XF, et al. [IL-15 enhance the therapeutic effects of whole cell vaccine in mouse CT26 colon carcinoma model]. Sichuan Da Xue Xue Bao Yi Xue Ban. 2010; 41: 567-570, 594.

31. Chen X, Ni J, Meng H, Li D, Wei Y, Luo Y, et al. Interleukinâ€'15: a potent adjuvant enhancing the efficacy of an autologous wholeâ€'cell tumor vaccine against Lewis lung carcinoma. Mol Med Rep. 2014; 10: 1828-1834.

32. Romano E, Rossi M, Ratzinger G, de Cos MA, Chung DJ, Panageas KS, et al. Peptide-loaded Langerhans cells, despite increased IL15 secretion and T-cell activation in vitro, elicit antitumor T-cell responses comparable to peptide-loaded monocyte-derived dendritic cells in vivo. Clin Cancer Res. 2011; 17: 1984-1997.

33. Steel JC, Ramlogan CA, Yu P, Sakai Y, Forni G, Waldmann TA, Morris JC. Interleukin-15 and its receptor augment dendritic cell vaccination against the neu oncogene through the induction of antibodies partially independent of CD4 help. Cancer Res. 2010; 70: 1072-1081.

34. Anguille S, Van Acker HH, Van den Bergh J, Willemen Y, Goossens H, Van Tendeloo VF, et al. Interleukin-15 Dendritic Cells Harness NK Cell Cytotoxic Effector Function in a Contact- and IL-15-Dependent Manner. PLoS One. 2015; 10: e0123340.

35. Anguille S, Lion E, Van den Bergh J, Van Acker HH, Willemen Y, Smits EL, et al. Interleukin-15 dendritic cells as vaccine candidates for cancer immunotherapy. Hum Vaccin Immunother. 2013; 9: 1956-1961.

36. Van den Bergh J, Willemen Y, Lion E, Van Acker H, De Reu H, et al. Transpresentation of interleukin-15 by IL-15/IL-15Ra mRNA-engineered human dendritic cells boosts antitumoral natural killer cell activity. Oncotarget. 2015; 6: 44123-44133.

37. Suzuki K, Nakazato H, Matsui H, Hasumi M, Shibata Y, Ito K, Fukabori Y. NK cell-mediated anti-tumor immune response to human prostate cancer cell, PC-3: immunogene therapy using a highly secretable form of interleukin-15 gene transfer. J Leukoc Biol. 2001; 69: 531-537.

38. Tasaki K, Yoshida Y, Miyauchi M, Maeda T, Takenaga K, Kouzu T, et al. Transduction of murine colon carcinoma cells with interleukin-15 gene induces antitumor effects in immunocompetent and immunocompromised hosts. Cancer Gene Ther. 2000; 7: 255-261.

39. Hazama S, Noma T, Wang F, Iizuka N, Ogura Y, Yoshimura K, et al. Tumour cells engineered to secrete interleukin-15 augment anti-tumour immune responses in vivo. Br J Cancer. 1999; 80: 1420-1426.

40. Jalah R, Rosati M, Kulkarni V, Patel V, Bergamaschi C, Valentin A, et al. Efficient systemic expression of bioactive IL-15 in mice upon delivery of optimized DNA expression plasmids. DNA Cell Biol. 2007; 26: 827-840.

41. Morris JC, Ramlogan-Steel CA, Yu P, Black BA, Mannan P, Allison JP, Waldmann TA. Vaccination with tumor cells expressing IL-15 and IL-15RÎ± inhibits murine breast and prostate cancer. Gene Ther. 2014; 21: 393-401.

42. Steel JC, Ramlogan-Steel CA, Morris JC. Genetically modified tumour cells expressing IL-15 and IL-15Ra as an in situ cancer vaccine platform. 9th Biennial Meeting of the Australasian-Gene-and-Cell-Therapy-Society. 2015.

43. Berger C, Berger M, Hackman RC, Gough M, Elliott C, Jensen MC, et al. Safety and immunologic effects of IL-15 administration in nonhuman primates. Blood. 2009; 114: 2417-2426.

44. Croci S, Nanni P, Palladini A, Nicoletti G, Grosso V. Interleukin-15 is required for immunosurveillance and immunoprevention of HER2/neu-driven mammary carcinogenesis. Breast Cancer Res. 2015; 17: 70.

45. Meazza R, Verdiani S, Biassoni R, Coppolecchia M, Gaggero A, Orengo AM, et al. Identification of a novel interleukin-15 (IL-15) transcript isoform generated by alternative splicing in human small cell lung cancer cell lines. Oncogene. 1996; 12: 2187-2192.

46. Tagaya Y, Kurys G, Thies TA, Losi JM, Azimi N, Hanover JA, et al. Generation of secretable and non-secretable interleukin-15 isoforms through alternate usage of signal peptides. Proc Natl Acad Sci USA. 1997; 94: 14444-14449.

47. Onu A, Pohl T, Krause H, Bulfone-Paus S. Regulation of IL-15 secretion via the leader peptide of two IL-15 isoforms. J Immunol. 1997; 158: 255-262.

48. Mattei F, Schiavoni G, Belardelli F, Tough DF. IL-15 is expressed by dendritic cells in response to type I IFN, double-stranded RNA, or lipopolysaccharide and promotes dendritic cell activation. J Immunol. 2001; 167: 1179-1187.

49. Yamaji K, Nabeshima S, Murata M, Chong Y, Furusyo N, Ikematsu H, Hayashi, et al. Interferon-alpha/beta upregulate IL-15 expression in vitro and in vivo: analysis in human hepatocellular carcinoma cell lines and in chronic hepatitis C patients during interferon-alpha/beta treatment. Cancer Immunol Immunother. 2006; 55: 394-403.