Interleukin-15 and Its Receptor Augment Dendritic Cell Vaccination against the neu Oncogene through the Induction of Antibodies Partially Independent of CD4 Help

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Abstract

Interleukin-15 (IL-15) stimulates the differentiation and proliferation of T, B, and natural killer cells; enhances CD8+ cytolytic T-cell activity; helps maintain CD4+CD8+ memory T cells; and stimulates immunoglobulin synthesis by B cells. IL-15 is trans-presented to effector cells by its receptor, IL-15Rα, expressed on dendritic cells (DC) and monocytes. We examined the antitumor effect of adenoviral-mediated gene transfer of IL-15 and IL-15Rα to augment a DC vaccine directed against the NEU (ErbB2) oncoprotein. Transgenic BALB-neuT mice vaccinated in late-stage tumor development with a DC vaccine expressing a truncated NEU antigen, IL-15, and its receptor (DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα) were protected from mammary carcinomas, with 70% of animals tumor-free at 30 weeks compared with none of the animals vaccinated with NEU alone (DCAd.Neu). The combination of neu, IL-15, and IL-15Rα gene transfer leads to a significantly greater anti-NEU antibody response compared with mice treated with DCAd.Neu or DCAd.Neu combined with either IL-15 (DCAd.Neu+Ad.mIL-15) or IL-15Rα (DCAd.Neu+Ad.mIL-15Rα). The antitumor effect was antibody mediated and involved modulation of NEU expression and signaling. Depletion of CD4+ cells did not abrogate the antitumor effect of the vaccine, nor did it inhibit the induction of anti-NEU antibodies. Coexpression of IL-15 and IL-15Rα in an anticancer vaccine enhanced immune responses against the NEU antigen and may overcome impaired CD4+ T-helper function. Cancer Res; 70(3); 1072–81. ©2010 AACR.

Introduction

Interleukin-15 (IL-15) is a pleiotropic cytokine that was identified for its ability to stimulate T-cell proliferation (1–3). It was further shown to induce the proliferation of natural killer (NK) cells, B cells, and IFN-producing killer dendritic cells (DC; refs. 4–6). IL-15 is essential for the differentiation and maintenance of memory CD8+ T cells, NK/T cells, and NK cells (7). It also stimulates immunoglobulin synthesis by B cells (5), promotes development of DC (8), and stimulates the production of proinflammatory cytokines by macrophages (9). IL-15 mRNA is expressed by a wide variety of cells, including monocytes, macrophages, DCs, fibroblasts, epithelial cells of various origins, and skeletal muscle (10). IL-15 itself has a short half-life and its expression is tightly regulated at the translational level (11).

IL-15 signals through a heterotrimeric receptor composed of the cytokine-specific IL-15Rα, IL-2R/IL-15Rβ (CD122) and γc, and IL-15Rγc, which results in the activation of various downstream signaling pathways. IL-15R is composed of the IL-15Rαα chain (12, 13). Unlike IL-2, which requires all three receptor subunits for high affinity binding, IL-15 is highly bound by IL-15R alone. Trans-presentation of IL-15 by IL-15Rα expressed on activated DC and monocytes to the IL-2R/IL-15Rβc and γc on effector T, B, and NK cells is thought to be the dominant mechanism for IL-15 action (14). Burkett and colleagues (15) showed the requirement for coexpression of IL-15 and IL-15Rα by nonlymphoid cells, such as DC, to support IL-15 function. IL-15Rα has been shown to stabilize IL-15 and increase the half-life of the cytokine (16–19). The similar pathology of IL-15Rα−/− and IL-15Rα−/− mice, and the ability of complexes of IL-15 with soluble IL-15Rα−IgFc to correct the immune defects in these mice, is evidence for the role of IL-15Rα in IL-15 action (16).

We examined the antitumor effect of a DC vaccine expressing IL-15, IL-15Rα, and the tumor antigen NEU in a transgenic mouse breast cancer model. Coexpression of IL-15 and IL-15Rα prevented or delayed development of mammary carcinomas in this aggressive late-stage tumorigenesis model. Furthermore, we showed that mice vaccinated with DC coexpressing IL-15 and IL-15Rα generated significantly greater levels of anti-NEU antibodies compared with DC expressing the tumor antigen alone or expressing the NEU antigen along with either IL-15 or IL-15Rα. Coexpression of IL-15 and IL-15Rα allowed for the induction of humoral immunity largely independent of CD4 help that may be an added benefit in the setting of reduced or dysfunctional CD4+ T cells as in patients with cancer or HIV infection.
Materials and Methods

Cell lines. The NEU-expressing cell lines TUBO and N202.1A derived from mammary cancers from a BALB-neuT and FVB-neuN mouse, respectively, were gifts from Dr. Patricia Nanni (University of Bologna, Bologna, Italy; refs. 20, 21) and were grown in DMEM (BioSource, Inc.) with 10% fetal bovine serum (FBS; Gemini) and 10 μg/mL gentamicin sulfate (BioSource). Human embryonic kidney (HEK-293) cells were grown in DMEM with 10% FBS and 10 μg/mL gentamicin sulfate (BioSource) and purchased from the American Type Culture Collection (ATCC).

Adenoviruses. The cDNA encoding the extracellular and transmembrane (ECM-tm) domains of the rat neu oncogene was provided by Dr. Augusto Amici (University of Camerino, Camerino, Italy; ref. 20). The murine IL-15 and IL-15Rα cDNAs (14) were provided by Dr. Yutaka Tagaya [National Cancer Institute (NCI), Bethesda, MD]. Ad.Neu, Ad.mIL-15, and Ad.mIL-15Rα are E1, E3-deleted recombinant adenoviruses (rAd) expressing the neu ECM-tm domains, murine IL-15, or IL-15Rα, respectively. Ad.null is an E1, E3-deleted rAd expressing no transgene. All vectors were generated using the AdMax system (Microbix; ref. 24), plaque isolated, expanded on HEK-293 cells, purified on two-step and continuous CsCl gradients or anion-exchange column (Sartorius Stedim), titered as plaque-forming units (pfu)/mL, and stored at −70°C.

Animals. Animal studies were approved by the Animal Care and Use Committee of the NCI. Female BALB-neuT mice, transgenic for a transforming rat neu oncogene under the control of a chimeric mouse mammary tumor virus promoter (25), were from a breeding colony established at the NCI. Female BALB/c mice were obtained from the Division of Cancer Treatment, NCI (Frederick, MD). CD4+ (H-2d) (28, 29). Briefly, 2 × 10^5 N202.1A cells were incubated with 20 units/mL IL-2 (PeproTech) for 5 d. Effector cells treated TUBO stimulator cells in RPMI 1640 supplemented with 20 units/mL IL-2 (PeproTech) for 5 d. Effector cells were assayed for their ability to lyse TUBO cells at E:T ratios of 100:1, 10:1, and 1:1. Cytotoxicity was quantified by lactate dehydrogenase release (CytoTox96 Non-Radioactive Cytotoxicity Assay, Promega) as per the manufacturer’s protocol. The percent cytotoxicity was calculated as follows: 100 × [(experimental release) – (effector spontaneous release) – (target spontaneous release)] / [(target maximum release) – (target spontaneous release)].

Immunological assays. Spleen cells were isolated 1 wk after the last vaccination with either DCAd.Neu or DCAd.Neu+Ad.mIL-15 as described previously (28). Briefly, 2 × 10^9 N202.1A cells were incubated with test sera diluted 1:10 in 1% FBS in PBS at 4°C for 1 h. Cells were washed and incubated with FITC-labeled rabbit anti-mouse immunoglobulin. IL-15Rα was detected by FITC-labeled anti-mouse IL-15Rα polyclonal antibody (R&D Systems) and analyzed using FlowJo software (Tree Star, Inc.).

Cellular response assays. To detect cytolytic responses, splenocytes were isolated 1 wk after the last vaccination with either DCAd.Neu or DCAd.Neu+Ad.mIL-15 as previously described (28, 29). Briefly, 2 × 10^9 N202.1A cells were incubated with test sera diluted 1:10 in 1% FBS in PBS at 4°C for 1 h. Cells were washed and incubated with FITC-labeled rabbit anti-mouse immunoglobulin antibody (DAKO) and mean fluorescence intensity was measured by flow cytometry.

Cell lines. The NEU-expressing cell lines TUBO and N202.1A derived from mammary cancers from a BALB-neuT and FVB-neuN mouse, respectively, were gifts from Dr. Patricia Nanni (University of Bologna, Bologna, Italy; refs. 20, 21) and were grown in DMEM (BioSource, Inc.) with 10% fetal bovine serum (FBS; Gemini) and 10 μg/mL gentamicin sulfate (BioSource). Human embryonic kidney (HEK-293) cells were grown in DMEM with 10% FBS and 10 μg/mL gentamicin sulfate (BioSource) and purchased from the American Type Culture Collection (ATCC).

Peptides. Synthetic peptides p66 (TYVYPANASL), a dominant rat neu epitope (22), HEX486-494 (KYSPSNVKI) from adenovirus hexon (23), and OVA257-264 (SIINFEKL) from hen ovalbumin (23) were purchased from GenScript.

Adenoviruses. The cDNA encoding the extracellular and transmembrane (ECM-tm) domains of the rat neu oncogene was provided by Dr. Augusto Amici (University of Camerino, Camerino, Italy; ref. 20). The murine IL-15 and IL-15Rα cDNAs (14) were provided by Dr. Yutaka Tagaya [National Cancer Institute (NCI), Bethesda, MD]. Ad.Neu, Ad.mIL-15, and Ad.mIL-15Rα are E1, E3-deleted recombinant adenoviruses (rAd) expressing the neu ECM-tm domains, murine IL-15, or IL-15Rα, respectively. Ad.null is an E1, E3-deleted rAd expressing no transgene. All vectors were generated using the AdMax system (Microbix; ref. 24), plaque isolated, expanded on HEK-293 cells, purified on two-step and continuous CsCl gradients or anion-exchange column (Sartorius Stedim), titered as plaque-forming units (pfu)/mL, and stored at −70°C.

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To detect a CD8+ response against the NEU antigen, splenocytes were assayed for IFN-γ secretion. Splenocytes from groups of animals (n = 3) vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs or DCAd.null were pooled and plated at 2 × 10⁶ cells per well in 24-well plates in triplicate. Splenocytes were cocultured with 10 μg/mL of the CD8 dominant peptides p66 (NEU), OVA257-264, or HEX486-494 for 72 h. Supernatants were collected and IFN-γ was measured by ELISA (R&D Systems) according to the manufacturer’s instructions. All samples were tested in triplicate.

**Adoptive serum transfer.** Serum collected from BALB/c mice immunized with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs or injected with PBS was pooled, diluted 3-fold, titered, and stored at 4°C. Groups of naive BALB/c mice were i.p. injected with 0.3 mL of the diluted serum every 3 d from day 5 to day 17 after s.c. injection of 1 × 10⁶ TUBO cells. Mice were examined twice weekly for tumor growth.

**Effects of immunized serum on NEU signaling and apoptosis.** Alteration of NEU (ErbB2), AKT, and p38 expression and phosphorylation following treatment with sera from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs–vaccinated mice was examined using a cell-based ELISA (30). TUBO cells were seeded at 1 × 10⁴ per well in a 96-well plate. The following day, the medium was exchanged for medium containing 5% FBS + 5% sera from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs–Vaccinated mouse or 5% FBS + 5% sera from PBS-injected mice. The cells were incubated for 0, 1, or 4 h, and NEU, AKT, and p38 phosphoprotein and total protein levels were quantitated using the Cellular Activation of Signaling ELISA (CASE kit, SuperArray Bioscience) following the manufacturer’s instructions.

Apoptosis was detected using a ssDNA Apoptosis ELISA kit (Chemicon International). TUBO cells were seeded at 1 × 10⁴ per well in a 96-well plate. The following day, medium was exchanged for medium containing 5% FBS + 5% sera from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs–Vaccinated mouse or 5% FBS + 5% sera from PBS-injected mice and incubated for 24 h. Apoptosis was detected according to the manufacturer’s protocol.

**Statistical analysis.** Statistical analysis was performed using JMP Statistical Software version 5.1 (SAS Institute, Inc.). Kaplan-Meier nonparametric regression analyses were performed for tumor prevention experiments with significance determined by the log-rank test. The comparison of the effect of vaccination on antibody titers among different groups was analyzed by one-way analysis using Tukey-Kramer honestly significant difference and nonparametric Wilcoxon/Kruskal-Wallis tests. A P value of <0.05 was considered significant.

**Results**

**DCs transduced with Ad.Neu, Ad.mIL-15, and Ad.mIL-15Ra expressed NEU (ErbB2) oncoprotein, IL-15, and IL-15Ra and exhibit maturation.** DCs from 10-day bone marrow cultures expressed CD80, CD86, CD40, and MHC class II. Compared with unmodified DC, DC transduced with Ad.Neu, Ad.mIL-15, and Ad.mIL-15Ra (DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rs) or Ad.Neu alone (DCAd.Neu) expressed higher levels of CD80, CD86, CD40, and MHC class II indicating maturation (Fig. 1A).

Bone marrow–derived DCs transduced with Ad.Neu together with Ad.mIL-15 and Ad.mIL-15Ra, Ad.Neu, or Ad.null at a MOI of 10 pfu/cell were examined by flow cytometry for DC maturation markers CD40, CD80, CD86, and MHC class II (A) and surface expression of NEU, IL-15, and IL-15Ra (B).

![Figure 1](cancerres.aacrjournals.org) DCs transduced with Ad.Neu, Ad.mIL-15, and Ad.mIL-15Ra exhibit a mature phenotype and express NEU, IL-15, and IL-15Ra. Bone marrow–derived DCs transduced with Ad.Neu together with Ad.mIL-15 and Ad.mIL-15Ra, Ad.Neu, or Ad.null at a MOI of 10 pfu/cell were examined by flow cytometry for DC maturation markers CD40, CD80, CD86, and MHC class II (A) and surface expression of NEU, IL-15, and IL-15Ra (B).

Forty-eight hours after transduction with Ad.Neu, or the combination of Ad.Neu, Ad.mIL-15, and Ad.mIL-15Ra, DCs were examined for the expression of NEU, IL-15, and IL-15Ra by flow cytometry (Fig. 1B). NEU protein was detected in DC transduced with Ad.Neu at comparable levels with those transduced with the combination of Ad.Neu, Ad.mIL-15, and Ad.mIL-15Ra. Expression was not observed in DC transduced with Ad.null. IL-15 was detected in DCAd.Neu+Ad.mIL-15+Ad.mIL-15Ra but not on DCAd.Neu or DCAd.null. IL-15Ra was detected on all DCs; however, greater expression levels were observed in DC transduced with Ad.Neu in combination with Ad.mIL-15 and Ad.mIL-15Ra.

**Vaccination of BALB-neuT mice in late-stage tumorigenesis with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Ra prevented autochthonous mammary cancers.** Mice treated with DCAd.Neu or DCAd.mIL-15+Ad.mIL-15Ra showed no survival advantage over mice treated with the control DCAd.null vaccine, with survivals of 17 and 18 weeks, respectively (P = 0.47 and
were examined twice weekly for the formation of mammary tumors. Creases in serum anti-NEU antibodies when compared with mice treated with triple vaccination of DCAd-null, DCAd.Neu, DCAd.Neu+Ad.mIL-15, or DCAd.Neu+Ad.mIL-15Rα showed improved tumor-free survival compared with DCAd.Neu (P = 0.014 and 0.005), possibly due to the effect of endogenous IL-15 and IL-15Rα interacting with the transferred receptor or cytokine, respectively. Mice receiving DCAd.Neu+Ad.mIL-15Rα had a median tumor-free survival of 22.5 weeks, and at 30 weeks, 10% of mice were free of tumor, whereas mice treated with DCAd.Neu+Ad.mIL-15Rα survived a median of 23 weeks with 20% of mice tumor-free at 30 weeks. Mice treated with the triple combination, DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα, exhibited significantly greater tumor-free survival when compared with mice treated with DCAd.Neu (P < 0.001), DCAd.Neu+Ad.mIL-15 (P = 0.001), DCAd.Neu+Ad.mIL-15Rα (P = 0.004), or DCAd.null (P = 0.001). Furthermore, in mice treated with the Ad. null-modified DC or DCAd.Neu, the onset of the first tumor occurred at 14 weeks, and all of the mice developed at least one mammary cancer by 23 weeks. In mice vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα, the first tumor occurred at 22 weeks and 70% of mice remained free of tumor at 30 weeks.

DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccination induced serum anti-NEU antibodies but not a CTL response. To determine the ability of the triple vaccination to induce cell- and antibody-mediated immune responses, we examined the induction of anti-NEU antibodies and tumor-specific CTL. Vaccination with DCAd.null or DCAd.mIL-15+Ad.mIL-15Rα failed to induce significant increases of anti-NEU antibodies compared with Ad.null-vaccinated mice (Fig. 3A). In contrast, mice treated with DCAd.Neu, DCAd.Neu+Ad.mIL-15, DCAd.Neu+Ad.mIL-15Rα, or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα showed significant increases in serum anti-NEU antibodies when compared with DCAd.null vaccination (P < 0.05; Fig. 3A). Furthermore, vaccination with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα induced on average 63%, 53%, and 102% greater levels of anti-NEU antibodies compared with mice treated with DC transduced with Ad.Neu alone. Compared with all other groups, mice vaccinated with DC transduced with the combination of Ad.Neu, Ad.mIL-15, and Ad.mIL-15Rα generated the highest titers of anti-NEU antibodies (P < 0.05). To investigate the functionality of the anti-NEU antibodies, we examined their ability to induce apoptosis of the neu+ TUBO cell line in vitro (Fig. 3B). Serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice induced apoptosis of TUBO cells. This effect was significantly greater than that seen with serum from PBS-treated mice. The induction of tumor cell apoptosis by anti-NEU antibodies may be a mechanism for the reduction in tumor formation in the BALB-neuT mice.

Tumor-specific CTL could not be shown in BALB-neuT mice vaccinated with DC expressing neu alone or in combination with IL-15 and/or IL-15Rα (Fig. 3C). When looking at IFN-γ secretion by the splenocytes of mice vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα versus DCAd.null restimulated with CD8-specific peptides for NEU, ovalbumin, or adenovirus hexon, we found little IFN-γ production in the NEU-vaccinated mice stimulated with the NEU-specific peptide (Fig. 3D). In contrast, when these splenocytes were stimulated with the adenovirus-specific peptide, there was a strong IFN-γ response detected. No IFN-γ secretion was detected when they were stimulated with the irrelevant OVA257-264 peptide.

Serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice inhibited tumor growth in vivo and decreased NEU (ErbB2) signaling in vitro. To explore whether NEU-specific antibodies played a role, we looked at whether vaccination would protect mice from tumor challenge with the neu-expressing TUBO tumor cell line. All the animals treated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα or PBS developed tumors within 7 days of challenge; however, in mice vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα, the tumors regressed, whereas in the PBS-treated mice the tumors continued to grow (Fig. 4A).

Next, we examined if serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice could inhibit tumor in naive mice challenged with TUBO cells. Serum from animals vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα inhibited the growth of TUBO tumors compared with mice receiving serum from the PBS-treated animals (P < 0.01; Fig. 4B). This indicates that specific anti-NEU antibodies play a protective role following vaccination.

To examine how anti-NEU antibodies inhibited tumor growth, we looked at NEU protein expression and signaling in TUBO cells following exposure to serum from immunized mice. Serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-Vaccinated mice induced time-dependent inhibition of NEU phosphorylation as well as reduced total levels of NEU protein. After 4-hour incubation with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα serum, the levels of phosphorylated NEU in TUBO cells were significantly lower than that detected at time 0 or...
when compared with incubation in PBS-treated mouse serum at any time point ($P < 0.05$). Total NEU levels were also reduced after 4 hours of incubation with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα–vaccinated serum, with average NEU levels 28% lower than that detected at time 0 ($P < 0.05$; Fig. 4C). After showing that the DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccine induced antibodies that can inhibit phosphorylation of NEU as well as downmodulate its overall level, we examined the effect on signaling in pathways downstream of NEU. Four hours after incubation with serum from mice vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα, TUBO cells showed reduced levels of phosphorylated AKT and increased phosphorylated p38 protein. At 4 hours, phosphorylated AKT was 43% lower than at time 0, whereas the level of phosphorylated p38 was 51% higher than at time 0, or when compared with the PBS-treated serum (Fig. 4D). Reduction in phosphorylated AKT expression and increased p38 phosphorylation are indicative of decreased NEU signaling (31).

**CD4+ T cells are required for antitumor immunity with DCAd.Neu vaccination but not DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccination.** To further explore the effect of the combination of IL-15 and its receptor on antitumor vaccination, immune cell subpopulations in groups of BALB/c mice were depleted. All mice receiving DCAd.null developed palpable tumors by 21 days after implantation (Fig. 5A and B). Animals vaccinated with DCAd.Neu or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα were protected from tumor (Fig. 5A and B). When mice were depleted of CD8+, CD4+, or NK cells after vaccination with DCAd.Neu or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα, they also failed to form tumors. However, mice depleted of CD4+ cells before DCAd.Neu vaccination all developed tumors, indicating a need for CD4+ T-cell help for an effective response. In contrast, mice depleted of CD4+ cells before DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccination were fully protected from tumors, indicating that CD4+ T-cell help may not be required when IL-15 and IL-15Rα were included in the vaccine (Fig. 5B).

DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccination induced anti-NEU antibodies in CD4-depleted mice but not in CD4-null mice. To determine if the triple vaccine was able to prime B lymphocytes to produce NEU-specific antibodies in the absence of CD4+ T help, we depleted CD4+ cells before vaccination and examined anti-NEU antibody levels. Significant levels of antibody were detected in the sera of DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα–vaccinated mice with a normal CD4 component as well as those depleted of CD4+ cells (Fig. 6A). In contrast, mice vaccinated with DCAd.Neu showed high levels of antibody when CD4+ cells were present; however, when CD4+ cells were depleted, DCAd.Neu–treated mice produced little antibody with vaccination.
These data indicate that exogenous IL-15 and IL-15Rα incorporated into DC vaccines may compensate for the need for CD4 help in priming B cells to produce antibody.

We further examined this effect using CD4−/− knockout mice. Unlike the antibody-mediated CD4 depletion model, CD4−/− mice vaccinated with either DCAd.Neu or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα showed only low levels of anti-NEU antibodies after vaccination (Fig. 6B). There was no difference in the levels of antibody produced whether the mice were given DCAd.Neu or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα. In addition, there was no protection afforded by either vaccine from the challenge with TUBO cells.

**Discussion**

IL-15 has shown the ability to increase the effectiveness of vaccines through enhancement of both innate and adaptive immune responses. Coexpression of its receptor, IL-15Rα, additionally enhances the biological activity of IL-15 through improved trans-presentation of IL-15 to the signaling β and common γ receptors (15, 17–19). In late-stage (12-week-old) BALB-neuT transgenic mice, vaccination with genetically modified DC expressing IL-15, IL-15Rα, and a truncated NEU antigen prevented or significantly delayed the onset of breast tumors (Fig. 2). At this age, the mice already show advanced microscopic mammary lesions (32) and immunization with DCAd.Neu alone offered little or no benefit.

Further examining the effects of vaccination with IL-15, IL-15Rα, and NEU, we found that this triple combination enhanced humoral immune responses to the NEU antigen compared with vaccination with NEU-expressing DC alone or when singularly combined with IL-15 or IL-15Rα (Fig. 3A). This is consistent with increases in antibody titers reported by others using IL-15 as an adjuvant combined with smallpox vaccination and with that observed with an experimental HIV vaccine in mice (33, 34). The isotypes observed following vaccination with...
DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα were largely IgG1 and IgG2a, with lesser amounts of IgG2b and IgM (data not shown). This pattern is consistent with other reports vaccinating with Ad.Neu alone (29). The addition of IL-15 and IL-15Rx did not change the relative predominance of isotypes but rather resulted in overall increased antibody levels. This increase may result from the ability of IL-15 to enhance proliferation, differentiation, and immunoglobulin synthesis by B cells (5, 35, 36). In addition, IL-15 also inhibits B-cell apoptosis (37) and may play a role in generating long-term serologic memory (36).

Subcutaneous vaccination either with DCAd.Neu or directly with Ad.Neu did not result in a measurable anti-NEU CTL response in our model (28, 29). Although IL-15 has been shown to increase CTL responses (33), we were unable to show classic CTL, or large increases in IFN-γ secretion (Fig. 3C and D). The antitumor response seemed to be largely due to induction of antibody. This was confirmed by the observation that serum transferred from Ad.Neu hypervaccinated mice protected naive mice from tumor challenge, indicating an inhibitory effect of NEU-specific antibodies (29, 38). Transfer of serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-immunized mice into naive TUBO-bearing mice inhibited tumor growth (Fig. 4B). Furthermore, IL-15 itself was undetectable in the serum of DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice (data not shown). As the antitumor effect occurred in the absence of IL-15, we concluded that these anti-NEU antibodies alone were sufficient to inhibit tumor growth. Passive immunization was not as effective as active vaccination with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα (Fig. 4A), likely due to limitations on the amount of antibody that could be transferred.

In vitro, serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice was able to inhibit phosphorylation of NEU as well as downmodulate NEU expression in TUBO cells (Fig. 4C). Serum from DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα-vaccinated mice also affected downstream NEU signaling pathways, decreasing activation of the promitotic AKT pathway and increasing p38 mitogen-activated protein kinase (MAPK) expression (Fig. 4D) that ultimately enhances apoptosis (Fig. 3B). These findings are consistent with previous studies that showed that anti-NEU antibodies from the serum of Ad.Neu-vaccinated mice (38) or the direct administration of monoclonal antibodies inhibited NEU phosphorylation and signaling (39). The anti-HER-2/neu monoclonal antibodies trastuzumab and pertuzumab used in the clinic inhibit breast cancer growth, in part, through activation of p38 MAPK and inhibition of AKT (40, 41).

CD4+ T cells are required for induction of antitumor responses in BALB-neuT mice (28, 29). Indeed, when DCAd.Neu alone was used to vaccinate mice, depletion of CD4+ cells before vaccination abrogated the antitumor effect (Fig. 5A). Depletion of CD4+ cells 72 to 96 hours after vaccination had no effect on the antitumor response (data not shown). This is consistent with a requirement for CD4+ help at the time of vaccination to facilitate B-cell priming (29). Depletion of CD8+ cells and NK cells before vaccination with either DCAd.Neu or DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα vaccination did not affect tumor-free survival, indicating that these cells do not play a critical role in antitumor immunity in this model (Fig. 5). Remarkably, mice depleted of CD4+ cells and vaccinated with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα showed no loss of antitumor response. Induction of an effective antitumor response in light of CD4+ cell depletion suggests that a lack of a normal CD4+ T-cell component may be overcome by the addition of IL-15 and IL-15Rx (Fig. 5B). Protective levels of antibody were produced with DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα despite the depletion of CD4+ cells, whereas mice vaccinated with DC lacking IL-15 and IL-15Rx did not produce adequate antibodies (Fig. 6A).

IL-15 increases proliferation and immunoglobulin secretion by B cells as well as upregulates expression of costimulatory molecules such as CD40L (42). In a study examining an experimental Pneumocystis vaccine in which CD40L was co-expressed, it was reported that antibodies to Pneumocystis were induced independent of CD4+ status (43).

Whereas the induction of antibodies in the absence of CD4+ help has not been reported for IL-15, the induction of CD8+ T-cell responses has (44, 45). Kutzler and colleagues (44) showed that when coadministering a vaccine with an

![Image](https://example.com/image.jpg)
optimized IL-15 expression plasmid, the resulting CD8+ T cells showed enhanced function and longevity that was largely independent of CD4+ help. Incorporation of CD40L into a vaccine was also found to enhance CD8+ T-cell responses in the absence of CD4+ help (46). In another study, Oh and coworkers (45) showed that, in the absence of CD4+ T-helper cells, antigen-specific T cells are short lived and exhibit defective secondary CD8+ T-cell responses because of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)–mediated apoptosis. Further, IL-15 codelivered with vaccines can largely overcome CD4+ T-cell deficiency of cytotoxic T cells by promoting longevity of antigen-specific CD8+ T cells and avoiding TRAIL-mediated apoptosis by modulating Bax and Bcl-xL expression (45). Similar upregulation of Bcl-xL and inhibition of apoptosis in B cells has also been reported following treatment with IL-15 (47). The ability of IL-15 to substitute for CD4+ help in the generation of CD8+ T-cell responses parallels the present study that showed that IL-15 can largely substitute for CD4+ T-cell help in the generation of antibody.

The ability of IL-15 with IL-15Rα to overcome a deficiency of CD4+ help is not absolute. The antibody response in CD4−/− mice was not mirrored in CD4 knockout mice. CD4−/− mice vaccinated with either DCAd.Neu+Ad.mIL-15+Ad.mIL-15Rα or DCAd.Neu did not produce significant levels of anti-NEU antibodies (Fig. 6B) and were not protected from challenge with tumor. These results are similar to that reported for the effects of IL-15 on CD8+ cells in the absence of CD4+ cells (44). The disparity in antibody production in animals depleted of CD4+ cells using antibody and CD4−/− mice suggests a fundamental difference in the CD4+ cell component of these models. Although the depletion model had CD4+ cell numbers reduced by >95%, it did retain a small population of CD4+ cells, whereas CD4−/− mice had no detectable CD4+ cells, suggesting that the combination of IL-15 and its receptor does require some, albeit at a reduced level, of CD4+ help for B-cell priming during vaccination.

The coexpression of IL-15 and IL-15Rα along with the NEU antigen in a genetically modified DC vaccine enhanced antitumor activity in late-stage mammary carcinogenesis in neu transgenic mice. The addition of IL-15 and IL-15Rα allowed induction of a protective antibody response against NEU in the setting of a severe deficiency of CD4+ T cells. This strategy may be valuable in patients deficient in CD4+ T-cell number or function, such as patients undergoing lymphocyte-depleting chemotherapy or suffering from advanced cancer (48) or those infected with HIV.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


41. Nahta R, Hung MC, Esteva FJ. The HER-2-targeting antibodies


Interleukin-15 and Its Receptor Augment Dendritic Cell Vaccination against the neu Oncogene through the Induction of Antibodies Partially Independent of CD4 Help

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