Interferon-α: An Effective Adjuvant for Peptide-Based Cytotoxic T-cell Vaccines

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Summary: An important issue for developing a vaccine therapy for human malignancy is identifying adjuvants that can elicit T-cell responses to peptides. The present study evaluates interferon-α (IFN-α) as a vaccine adjuvant. C57BL/6 mice were immunized subcutaneously with peptide derived from influenza virus (Flu) either with or without IFN-α using different vaccine formulations. IFN-α significantly enhanced cytotoxic T lymphocytes (CTL) induction in mice immunized with Flu peptide in incomplete Freund’s adjuvant (IFA). Flu peptide administered continuously for 3 days by osmotic pump with IFN-α could elicit CTL induction, whereas either Flu peptide or IFN-α alone was non-immunogenic. Furthermore, injection of the liquid formation of Flu peptide with IFN-α in phosphate-buffered saline (PBS) did not elicit CTL induction. These results suggest that the continuous administration of peptide and local delivery of IFN-α are important for efficient CTL induction, and that IFN-α is an effective adjuvant for peptide-based vaccines.

Key words interferon-α, adjuvant, OIL, vaccine

INTRODUCTION

Recent advances of molecular biology and cellular immunology in the field of tumor immunology have resulted in the identification of a large number of antigenic epitopes recognized by CTL reacting to tumor cells [1-4], thereby opening the door to new peptide-based specific immunotherapy of cancer. The use of synthetic peptides as immunizing antigens in cancer vaccines offers many advantages: relatively easy construction and production, chemical stability, and a lack of infectious or oncogenic potential [5]. Perhaps the most important advantage is the theoretical ability to manipulate the immune response through the use of defined antigenic epitopes. The limitation of this approach is that most peptides are weak immunogens. Peptide immunizations have elicited specific immune responses when injected with powerful adjuvants in animals, but many of these adjuvants are quite toxic for repeated use in humans [6,7]. The only adjuvants available for general use in humans are aluminium salts. However aluminium salts are poor inducers of Th1 and CTL responses. The use of cytokines to enhance immune response to vaccines is an area of growing interest. Recent studies have shown GM-CSF and IL-12 to be effective with peptide-based vaccines [8,9]. IFN-α has multiple biological activities including antiviral, antiproliferative, and immunological effects. IFN-α is the most frequently used cytokine in the treatment of some viral disease and cancer. In this study, using an in vivo mouse model, we show that IFN-α is an effective adjuvant for peptide-based vaccines.

MATERIALS AND METHODS

Animals and cell lines

Female C57BL/6 (H-2b) mice were obtained from Charles River Japan, and routinely used at 8-12 weeks of age. EL4 (C57BL/6, H-2b thymoma) was cultured in RPMI1640 (GIBCO BRL) with 10% FCS (Filtron), 100 U/ml penicillin (GIBCO BRL) and 100
µg/ml streptomycin (GIBCO BRL).

Peptide and IFN-α

Influenza virus (Flu) nucleoprotein-derived peptide with H-2Dβ-binding motif (ASNENMETM, Flu366-374) was synthesized by Fmoc methods, and the purity was 95-98%. The peptide was dissolved in PBS. Murine IFN-α was kindly provided by Sumitomo Pharmaceuticals, Osaka, Japan. Murine IFN-α was produced by infecting EAT cells, previously treated with sodium butyrate, with Newcastle disease virus and treating the infected cells with theophylline. IFN-α was purified on CPG column and anti-IFN-α antibody column. The biological activity of IFN-α was determined by the measurement method with inhibition of viral cytopathic effect (CPE) as an index.

Vaccine preparations and immunizations

Three different vaccine formulations were prepared as follows. (1) The peptide solution was mixed with incomplete Freund’s adjuvant (IFA) (WAKO Chemical) in connecting 1-ml glass syringes at 1:1 on a vol/vol to form a water-in-oil emulsion. (2) An osmotic pump (type 1003D. Alza) was used for continuous administration for 3 days. The pump was filled with peptide solution and implanted subcutaneously according to the instructions by the manufacturer. (3) The peptide in PBS was used as a liquid formation. Mice (n=3) were immunized subcutaneously by injecting 100 µg of peptide in the base of the tail.

Induction of activated spleen cells by in vitro stimulation of precursor CTL

Spleens of immunized mice were harvested at day 7 of immunization, mashed into a single cell suspension, and plated at 5×10⁶ cells/well in 24-well tissue culture plates. These cells were co-cultured with 5×10⁴ irradiated (3000 rad) syngeneic spleen cells, which had been pulsed with 90 µg/ml of Flu peptide for 1 h. Cultures were incubated for 5 days in RPMI 1640 with 10% FCS (Filtron), 10 mM glutamine, 50 µM 2-mercaptoethanol, 1 mM sodium pyruvate, and 0.1 mM non-essential amino acids (all supplements except for FCS were obtained from GIBCO BRL).

Cytotoxicity assay

The CTL activity of in vitro activated spleen cells was measured using a standard ⁵¹Cr release assays. Target cells were EL4 cells pulsed during ³⁵Cr labeling with 50 µg/ml peptide for 1 h. Serial dilution of effector cells and 1×10⁴ target cells were incubated for 4 hs. The percentage of specific lysis was calculated as 100×(release by CTL — spontaneous release)/(maximal release — spontaneous release). Spontaneous release in the absence of CTL was less than 20% of maximal release by detergent in all experiments.

RESULTS

IFN-α enhances CTL induction in mice immunized with Flu peptide in IFA

Mice were immunized s.c. (base of tail) with Flu peptide mixed with IFN-α in IFA. Flu peptide in IFA elicited CTL activity, and IFN-α (1×10⁵ U/mouse) enhanced the level of CTL activity compared with that in the control mice immunized with Flu peptide alone (Fig. 1). IFN-α (1×10⁴ U/mouse) failed to enhance CTL induction (Fig. 1).

IFN-α elicited CTL induction in mice immunized with Flu peptide by osmotic pumps

The osmotic pump delivers solution continuously and locally for 3 days at the implanted site. IFN-α

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**Figure 1.** IFN-α-enhanced CTL response to peptide immunization in IFA. Mice were immunized s.c. with 100 µg of Flu peptide and the indicated dose of IFN-α in IFA. Seven days later, spleen cells pooled from three mice were stimulated in vitro with Flu peptide-pulsed irradiated spleen cells and tested after 5 days for specific cytolytic activities against Flu peptide-pulsed EL4. Values represent the mean of triplicate cultures. Lysis of EL4 without Flu peptide was <2% (data not shown).
IFN-α AS A VACCINE ADJUVANT

(1 × 10⁵ U/mouse) elicited CTL induction in mice immunized with Flu peptide by osmotic pumps, whereas mice immunized with Flu peptide alone or IFN-α alone by osmotic pumps did not show any CTL activity (Fig. 2). IFN-α (1 × 10⁵ U/mouse) injected s.c. twice (at day 1 and 2) at the site of the osmotic pump, which had been filled with Flu peptide solution, could elicit CTL induction, whereas the CTL

Fig. 2. IFN-α-induced CTL response to peptide immunization by osmotic pumps. Mice were immunized s.c. continuously for 3 days by osmotic pumps with 100 μg of Flu peptide or 10⁵ U of IFN-α or 100 μg of Flu peptide plus 10⁵ U of IFN-α. As a positive control mice were immunized with 100 μg of Flu peptide in IFA. Seven days later, spleen cells pooled from three mice were stimulated in vitro with Flu peptide-pulsed irradiated spleen cells and tested after 5 days for specific cytolytic activities against Flu peptide-pulsed EL4. Values represent the mean of triplicate cultures. Lysis of EL4 without Flu peptide was <5% (data not shown).

Fig. 3. Effect of IFN-α on CTL response in mice immunized with Flu peptide. Mice were immunized with 100 μg of Flu peptide by osmotic pumps, 100 μg of Flu peptide plus 10⁵ U of IFN-α by osmotic pumps, or 100 μg of Flu peptide by osmotic pumps plus 10⁵ U of IFN-α injected twice (day 1 and 2) to the site of osmotic pumps. Seven days later, spleen cells pooled from three mice were stimulated in vitro with Flu peptide-pulsed irradiated spleen cells and tested after 5 days for specific cytolytic activities against Flu peptide-pulsed EL4. Values represent the mean of triplicate cultures. Lysis of EL4 without Flu peptide was <5% (data not shown).

Fig. 4. Effect of IFN-α on CTL response in mice immunized with Flu peptide in PBS. Mice were immunized with 100 μg of Flu peptide plus 10⁵ U of IFN-α by injections (day 0), or 100 μg of Flu peptide plus 10⁵ U of IFN-α by injections (day 0, 1, and 2). As a positive control mice were immunized with 100 μg of Flu peptide in IFA. Seven days later, spleen cells pooled from three mice were stimulated in vitro with Flu peptide-pulsed irradiated spleen cells and tested after 5 days for specific cytolytic activities against Flu peptide-pulsed EL4. Values represent the mean of triplicate cultures. Lysis of EL4 without Flu peptide was <2% (data not shown).
activity was lower than that of mice immunized with Flu peptide and IFN-α by osmotic pumps (Fig. 3).

**IFN-α did not elicit CTL induction in mice immunized with Flu peptide in PBS**

We next examined the effect of IFN-α on CTL induction when mice were immunized with Flu peptide in a liquid formation. IFN-α (1 × 10^5 U/mouse) could not elicit CTL response in mice after single (at day 0) or three (at day 0, 1, 2) immunizations in combination with Flu peptide in PBS (Fig. 4).

**DISCUSSION**

Our results demonstrate that local administration of IFN-α to the site of peptide inoculation was effective on the induction of peptide-specific CTL (Figs 1, 2 and 3). The local administration of IFN-α with antigen has the advantage of a lower risk of side effects than systemic administration. The effects of IFN-α on the immune system include activation of natural killer cells, stimulation of CD8+ T lymphocyte proliferation and survival, promotion of CD4+ Th1 cell differentiation, up-regulation of class I major histocompatibility complex (MHC) expression, and promotion of dendritic cell differentiation [10,11]. Vaccination studies with genetically modified tumor cells, secreting endogenous IFN-α, have shown that tumor cells expressing IFN-α are capable of eliciting systemic antitumor immunity [12]. Although these studies suggest the possibility that IFN-α acts as an adjuvant for CTL vaccines, there are very few reports about in vivo effects of IFN-α on CTL induction. Biological activity of IFN-α is species specific; for instance, human IFN-α has only low activity on mouse cells and vice versa, so we prepared murine IFN-α and used it in this in vivo study. To our knowledge, this is the first report that demonstrates directly in vivo that IFN-α is an effective adjuvant for peptide-based vaccines. Flu peptide administered continuously by osmotic pump while IFN-α was injected at the site of the peptide inoculation, elicited CTL response, whereas Flu peptide in PBS administered by injection with IFN-α did not (Figs 3 and 4). These results indicated that the peptide is weakly immunogen and the mode of administration of peptides is of critical importance in peptide-based vaccines. IFN-α has been used extensively in humans and has shown minimal toxicity even after repeated injections. The use of IFN-α in humans, as an adjuvant for peptide-based vaccines, may allow the realization of effective and long-lived immune response to immunogenic epitopes.

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**REFERENCES**


