Granulocyte-Macrophage Colony-Stimulating Factor: An Effective Adjuvant for Protein and Peptide-Based Vaccines

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The current studies evaluate granulocyte-macrophage colony-stimulating factor (GM-CSF) as a vaccine adjuvant. An important issue for developing vaccine therapy for human malignancy is identifying adjuvants that can elicit T-cell responses to proteins and peptides derived from "self" tumor antigens. GM-CSF, in vitro, stimulates the growth of antigen-presenting cells such as dendritic cells and macrophages. Initial experiments examined whether GM-CSF injected into the skin of rats could affect the number or character of antigen presenting cells, measured as class II major histocompatibility complex expressing cells, in lymph nodes draining the injection site. Intradermal (id) inoculation of GM-CSF every 24 hours for a total of five inoculations resulted in an increase of class II+ expressing cells that peaked at the fourth inoculation. Subcutaneous (sq) inoculation resulted in an increase of class II+ expressing cells that peaked following the second inoculation, then decreased over time. Using this schema for "conditioning" the inoculation site, GM-CSF was administered id or sq for five inoculations and a foreign antigen, tatanus toxoid (tt), was given at the beginning or the end of the immunization cycle. Id immunization was more effective than sq at eliciting tt specific immunity. In addition, GM-CSF id, administered as a single dose with antigen, compared favorably with complete Freund's adjuvant (CFA) and alum in eliciting tt specific antibody and cellular immunity. We have shown that immunity to rat neu (c-erbB-2) protein, an oncogenic self protein, can be generated in rats by immunization with peptides derived from the normal rat neu sequence plus CFA. The current study demonstrates that rat neu peptides inoculated with GM-CSF could elicit a strong delayed type hypersensitivity reaction (DTH) response, whereas peptides alone were non-immunogenic. GM-CSF was as effective as CFA in generating rat neu specific DTH responses after immunization with a new peptide based vaccine. Soluble GM-CSF is a potent adjuvant for the generation of immune responses to foreign proteins as well as peptides derived from a self tumor antigen.

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nicity. The increased immune response is presumed to be mediated by the role GM-CSF plays in the maturation and function of antigen presenting cells such as dendritic cells and macrophages. Further studies, using GM-CSF encapsulated in microspheres mixed with irradiated tumor cells and injected subcutaneously also demonstrated increased tumor immunogenicity. These encouraging studies with whole tumor cell preparations led us to question whether GM-CSF could be useful in augmenting specific antigen responses to soluble protein and peptides. We present an immunization strategy using GM-CSF as an adjuvant to increase the immunogenicity of antigen in both a foreign antigen and tumor antigen system.

MATERIALS AND METHODS

Animals. Rats used in this study were Fischer strain 344 (CDF (F-344)/CrjBR) (Charles River Laboratories, Portage, MI). Animals were maintained at the University of Washington Animal Facilities under specific pathogen free conditions and routinely used for experimental studies between 3 and 4 months of age.

Antigens/adjuvant. Experiments presented here evaluate GM-CSF as an adjuvant in two different antigen systems in rats: a foreign antigen, tetanus toxoid (tt); and peptides derived from a self antigen, rat neu protein. Preservative free tetanus toxoid (Lederle, American Cyanamid Corporation, Pearl River, NY) was diluted in sterile phosphate-buffered saline (PBS) and stored in aliquots at −70°C. Nine peptides derived from the amino acid sequence of rat neu were constructed, 5 from the ECD of the protein and 4 from the ICD of the protein. ECD peptides were p45-59, p98-112, p323-337, p332-349, and p343-447. ICD peptides were p781-795, p788-802, p932-948, and p1171-1185. These peptides, 15 to 20 amino acids in length, were shown in previous studies to elicit T-cell immune responses in Fischer rats when injected subcutaneously (sq) with a classical adjuvant, complete Freund’s adjuvant (CFA). The peptides were synthesized and purified by H. Zebrowski (University of Washington, Seattle, WA), then dissolved in PBS, pH 7.4, to give 2 mg/mL stock solutions. Before aliquoting, peptides were sterile filtered, then stored at −70°C. Two single peptides that could elicit peptide-specific T cells, p45-49 and p788-802 were used in preliminary studies. Multiple peptide combinations were used as immunizing antigens in later studies. Purified recombinant murine GM-CSF (kindly supplied by Immunix Corporation, Seattle, WA) was used as an adjuvant and a dose of 5–10 µg per inoculation was used.

Immunizations. Preliminary experiments defined the need to prepare the inoculation site with GM-CSF, theoretically to stimulate Langerhan’s cell growth and differentiation. Initial injections with either GM-CSF or PBS without an antigen to access immune cell trafficking were performed over five cytokine inoculations, each 24 hours apart. Rats’ backs were shaved and a template drawn to ensure inoculation would be at the same site each day. In the groups that received GM-CSF, the cytokine was administered at a dose of 10 µg per injection in a total volume of 50 µL. Animals in the control group received injections of 50 µL of sterile PBS. Groups received adjuvant intradermally (id) or sq. Each group consisted of three experimental animals (GM-CSF) and two control animals (PBS).

Groups of animals were killed 24 hours after 2, 3, 4, and 5 inoculations. Lymph nodes draining the injection site were analyzed for infiltrating cells. Animals showed no adverse effects to repeated injection at the same site.

Subsequent immunization protocols with antigen were based on the skin preparation strategy defined above. Animals were immunized with GM-CSF or PBS at the same site for five inoculations. In the tetanus experiments, tt was mixed with the adjuvants at a 1:5,000 dilution in

concentration of 3 Lf units per animal and administered with the first two injections or the last two injections. Animals were assessed for tt specific delayed type hypersensitivity reaction (DTH) responses, as an in vivo measure of cell mediated immunity, 20 days after the fifth injection. Serologic responses were evaluated 24 days after the fifth injection. In the experiments evaluating peptide specific responses, 250 µg of the rat neu peptides were administered with the first two of five cytokine or PBS injections. As with the tt immunizations, the peptides were mixed with the GM-CSF or PBS at the time of injection and inoculated together. Peptide specific DTH responses were assessed 20 days after the fifth injection. Immunizations were administered either id or sq.

The final series of experiments examined the necessity of multiple dose GM-CSF administration by evaluating immunization with a single dose of GM-CSF mixed with antigen, similar to the use of a “classical” adjuvant. These experiments also compared GM-CSF, as an adjuvant, with two other standard adjuvants, CFA (Sigma Immunochemicals, St Louis, MO) and Alum (Pierce, Rockford, IL). Tetanus toxoid was mixed with the adjuvants (5 µg GM-CSF, 1:1 with CFA, and 1:1 with Alum) and administered as a one time immunization. CFA and Alum with tt were administered sq. GM-CSF with tt was administered id or sq. Animals were assessed for DTH response and antibody immunity as described. Studies of a multiple peptide vaccine for the generation of rat neu specific immunity demonstrated that T-cell immunity could be generated with three in vivo immunizations with immunogenic rat neu peptides in CFA. Experiments described here duplicate these studies comparing CFA with GM-CSF as an adjuvant. Animals were inoculated with multiple peptides vaccines (mix of 5 rat neu ECD peptides or a mix of 4 rat neu ICD peptides at 100 µg/peptide) in CFA (sq) or 5 µg GM-CSF (id) once every 20 days for three immunizations. Twenty days after the third immunization, DTH responses were measured as described.

Flow cytometric analysis. Cells derived from the draining lymph nodes of rats injected id or sq with GM-CSF or PBS without any antigen were stained in suspension with MRC-OX-6 (Rat Ia; Serotec Ltd, Oxford, UK). Briefly, cells were harvested, washed, and resuspended in PBS-1% bovine serum albumin (BSA) and seeded into microtiter plates at a final concentration of 2 to 3 × 10^6 cells per well. Cells were incubated on ice with the unconjugated monoclonal antibody at saturating concentrations for 45 minutes, washed twice with 150 µL PBS-1% BSA and stained with FITC conjugated F(ab’)_2 rabbit antimouse IgG for an additional 30 minutes as the second step antibody. The second step FITC conjugate has been shown to have reduced cross reaction with rat (Serotec). Finally, all cells were treated by washing twice with PBS-1% BSA (Sigma Chemical Co, St Louis, MO) and fixed with 1% paraformaldehyde. Negative controls consisted of cells labeled with the second step antibody alone. Fluorescence analyses were performed by flow cytometry. Two micron fluorescein calibration grade microspheres (Polyscience, Warringtn, PA) are run on a daily basis to standardize the instrument.

ELISA for rat tetanus specific antibody responses. 96-well Immulon 4 plates (Baxter SP; Dynatech Laboratories, Redmond, WA) were incubated overnight at 4°C with tt diluted 1:1,000 in carbonate buffer (equimolar concentrations of Na_{2}CO_{3} and NaHCO_{3}, pH 9.6) alternating rows with buffer alone. After incubation, all wells were blocked with PBS-1% BSA, 100 µL per well for 1 hour at room temperature. The plate was washed with PBS-0.5% Tween and experimental sera was added at the following dilutions: 1:25 to 1:1,200 or 6,400. The sera was diluted in PBS-1% BSA-1% FBS-25 µg/mL mouse IgG-0.01% NaN_3 and then serially into PBS-1% BSA. Fifty microliters of diluted sera was added per well and incubated for 1 hour at room temperature. Each experimental sera was added to a well with tt and a well without tt. Sheep antirat Ig F(ab’)_2 horseradish peroxidase (HRP) was added to the wells at a 1:5,000 dilution in
PBS-1% BSA and incubated for 45 minutes at room temperature (Amersham Co, Arlington Heights, IL). Antibody class assays were performed similarly with rabbit antirat IgG F(ab')2 and sheep antirat IgG F(ab')2, antibodies as the second step antibody at a concentration of 1:5,000 (Serotec). Following the final wash, TMB (Kirkegaard and Perry Laboratories, Gaithersburg, MD) developing reagent was added. Tetanus toxoid immune control rat sera was derived by immunizing rats with tt 3 LFU and CFA/IFA for a priming immunization and two boosts each 20 days apart. This positive control rat sera was analyzed on each plate. When the optical density (OD) at 650 nm reached 0.3 at the 1:100 sera dilution of the "hyperimmunized" sera, the plate was developed. The mean and standard deviation of all controls in each experimental group show the interassay variability. Color reaction was read at an OD of 450 nm. The OD of each serum dilution was calculated as the OD of the tt coated wells minus the OD of the PBS-1% BSA coated wells. Sera derived from two nonimmunized animals was run on each plate as an assessment of native tt response. Antigen specificity was confirmed by analyzing experimental sera for antibody responses to ova albumin. In these analyses, plates were incubated overnight at 4°C with purified ova albumin at 10 μg/mL concentration in carbonate buffer alternating with rows of buffer alone. Antibody evaluation then proceeded as is described for tetanus.

**DTH response.** Change in ear thickness on exposure to an antigen is a measurement of DTH response in rodents.² Twenty days after the final inoculation baseline ear thickness was measured in each animal using a dual thickness gauge (Mitutoyo Corporation, Japan). Immediately following the baseline measurement, the left ear was treated epicutaneously with a carrier solvent consisting of a 1:1 mix of acetone and dibutyl pthalate. Ten microliters of the carrier solvent was applied to the front of the ear and 10 μL was applied to the back of the ear. The right ear of each animal was treated with the carrier solvent and antigen with 10 μL of the carrier diluent antigen mix applied to the front of the ear and 10 μL applied to the back of the ear. Animals in the tt immunization series were tested with 0.1 Lf U/mL of tt, animals immunized with the single rat neu peptide, either p45 or p788, and animals immunized with multiple rat neu peptides were tested with 1 μg/mL of purified rat neu protein. DTH response as a measure of ear thickness was measured at 48 hours and calculated as the difference in the thickness of the experimental ear compared with control.

**RESULTS**

**Subcutaneous or intradermal injection of GM-CSF for five inoculations increases the percent of class II MHC+ cells in regional lymph nodes.** Initial experiments examined whether local application of GM-CSF into a site where antigen would be introduced would be effective in the recruitment of cells with the potential ability to generate an immune response. Sq and id administration was studied. Class II MHC+ cells were used as a marker for the presence of APC. GM-CSF is known to upregulate class II surface expression on APC such as dendritic cells (DC) and macrophages.⁴⁻¹⁵ Cytokine was administered by both routes every 24 hours for a total of five inoculations to assess the length of time needed to recruit APC or affect the ability of APC to process antigen. Daily administration of GM-CSF increased the percent positive class II MHC expressing cells in regional lymph nodes. The results with id and sq administration differed (Fig 1). GM-CSF id resulted in a marked increase in fluorescence of class II expressing cells in regional lymph nodes later in the injection cycle, after four inoculations (A). This is shown for a representative animal from each group in Fig 2. GM-CSF sq induced an increase in class II expressing cells in draining lymph nodes early, within two injections, but after four injections was no different from control (B). Figure 3, FACS profiles from representative animals, demonstrated GM-CSF sq resulted in an early increase in class II expressing cells, which resolved over time. Animals immunized with PBS alone were used as a control. The total number of harvested cells from the draining lymph nodes from GM-CSF or PBS inoculated animals was not significantly different.

**Intradermal injection of GM-CSF for five inoculations is...**
an effective adjuvant for eliciting antibody responses to tetanus toxoid when the antigen is administered with the last two injections of cytokine. GM-CSF administration had an effect on class II expressing cells in regional lymph nodes. The augmentation of class II expressing cells was more marked in the id immunized animals and occurred later in the inoculation cycle. To determine whether this effect translated into an increased ability to induce an immune response, rats were inoculated with GM-CSF id versus sq. Tetanus toxoid was injected concurrently with the last two GM-CSF inoculations. All animals who received GM-CSF id with tt developed significant antibody responses compared with PBS controls (Fig 4). Anti-tt antibody titers of animals receiving sq immunization were lower (Fig 4). No antibodies were detected with the negative control antigen, ova albumin.

**Intradermal injection of GM-CSF for five inoculations is an effective adjuvant for eliciting antibody responses to tt when the antigen is administered with the first two injections of cytokine.** To determine whether adding antigen only after the site has been "conditioned" with GM-CSF is necessary, or whether co-administration of antigen and cytokine early in the cycle would suffice, rats were inoculated with GM-CSF id versus sq, with tt injected concurrently with the first two of five adjuvant inoculations. All six animals in the id GM-CSF adjuvant group developed a marked tt specific antibody response (Fig 5). None of the animals in the id PBS group developed vigorous antibody responses to tt. Animals who were immunized by the sq route developed anti-tt antibodies, but of a lower titer than the id immunized animals (Fig 5). No antibodies were detected with the negative control antigen, ova albumin. Prolonged "conditioning" of the vaccination site with GM-CSF before the introduction of antigen did not appear to be critical in the generation of an immune response. In both regimens, antigen administered late (Fig 4) or early (Fig 5), the id route of immunization appeared to be the most effective in generating significant anti-tt antibody responses. The remainder of the experiments concentrated on GM-CSF and antigen administered id as the optimal route of vaccination.

**Intradermal GM-CSF, as an adjuvant, elicits IgG antibody responses to tt.** Class of antibody elicited was analyzed for animals immunized with GM-CSF or PBS id for five inoculations. Rats were inoculated with 10 μg of GM-CSF id each day for five inoculations, each 24 hours apart. A control group was injected with PBS. Draining lymph nodes were analyzed 24 hours after 2, 3, 4, and 5 injections for class II MHC expression by flow cytometry. FACS analysis from a representative animal in each group is shown here.
Data from analysis of sera from an animal with high responses. In experiments depicted in Fig 5, animals that were not immunized are shown as an example of a native rat response to tetanus. Twenty-four days after the fifth inoculation, sera was obtained from each animal. Tetanus toxoid responses were determined by ELISA. Results are depicted as the mean and standard deviation of all experimental plates in this analysis.

GM-CSF, as an adjuvant, elicits DTH responses to tt similar to those seen in animals immunized with a standard adjuvant. Cell mediated immunity was assessed in the animals previously described by the measurement of tt specific DTH. All animals immunized with an adjuvant developed DTH responses to tt. GM-CSF immunized animals had responses similar to those seen in animals immunized with a standard adjuvant and tt (Fig 8). Animals immunized with PBS and tt developed weak to no DTH response.

Intradermal GM-CSF is a potent adjuvant for the generation of an immune response to peptides derived from a self tumor antigen. Experiments previously described show that GM-CSF id is an effective adjuvant for the generation of immune responses to foreign antigens. We questioned whether the same regimen would be as effective in augmenting immune responses in tumor antigen systems with peptide based vaccines. In initial experiments, two peptides derived from the rat neu protein sequence, p45 and p788, were chosen for analysis. Peptide p45 is derived from the extracellular domain of the neu protein and p788 is derived from the intracellular domain. This animal system, a model
Fig 6. Intradermal GM-CSF, as an adjuvant, elicits IgG antibody responses to tt. Rats were injected with GM-CSF or PBS id for five inoculations. tt was added to the cytokine or PBS inoculation at a concentration of 3 Lf units during the first two inoculations. An example of antibody class analysis is shown here for three GM-CSF and three PBS immunized animals. A control animal that was not immunized is shown as an example of a native rat response to tetanus. Twenty-four days after the fifth GM-CSF or PBS inoculation, sera was obtained from each animal. Tetanus toxoid responses were determined by ELISA. Results are shown at a sera dilution of 1:100. (A) The predominant IgG antibody responses of animals immunized with tt and GM-CSF id as adjuvant. (B) The responses of the PBS immunized and control animal.

for evaluating the generation of immunity to the HER-2/neu oncogene protein product in human adenocarcinomas, allows the development of an immune response to rat neu, a self protein, when CFA is used as an adjuvant.12 Animals immunized with p42 and p788 in CFA will generate peptide specific T-cell responses (M.L.D., unpublished observations, 1995). Initial experiments evaluated the 5-day regimen of GM-CSF inoculation with antigen administered on the first 2 days of the cycle. Id administration of GM-CSF for five inoculations with peptides administered with the first two injections induced vigorous peptide specific DTH responses (Fig 9A). Nonexistent or weak peptide specific responses were measured in rats who received peptide in PBS.

Protein specific rat neu cellular immune responses could be induced with a vaccine composed of multiple neu derived peptides administered with CFA sq after three in vivo immunizations.12 We compared this regimen with rat neu peptides inoculated id with GM-CSF. Vaccines consisting of 5 ECD peptides or 4 ICD peptides were administered once every

Fig 7. GM-CSF, administered in the same manner as a “classical” adjuvant, is effective in eliciting antibody responses to tt. Rats were injected with CFA sq, Alum sq, GM-CSF id or sq (5 µg) and PBS sq with tt at a concentration of 3 Lf units. Immunizations were administered on one day only with no repeated administration of GM-CSF. Six animals were included in each experimental group. This figure represents data collected from two separate experiments. Three control animals that were not immunized are shown as an example of a native rat response to tt. Twenty-four days after the immunizations sera was obtained from each animal. Tetanus toxoid responses were determined by ELISA. Results are depicted as the mean and standard deviation of the antibody response of each experimental group at each sera dilution. Positive control sera from an animal with high tt specific antibody was run on each plate and is expressed as the mean and standard deviation of all experimental plates in this analysis.

Fig 8. GM-CSF, as an adjuvant, elicits DTH responses to tt similar to those seen in animals immunized with a standard adjuvant. Rats were injected with CFA sq, Alum sq, GM-CSF id or sq (5 µg) and PBS sq with tt at a concentration of 3 Lf units. Immunizations were administered on one day only with no repeated administration of GM-CSF. Six animals were included in each experimental group. This figure represents data collected from two separate experiments. Twenty days after immunization, a DTH response to tt was measured in the immunized rats. Antigen was applied to rat ear and responses measured at 48 hours. Ear swelling of experimental compared with control ear was measured. Results are depicted as the mean and standard deviation of measurements taken from each experimental group.
A vaccine composed of a group of 5 ECD peptides or 4 ICD peptides taken from three animals in each experimental group. pg) was added to adjuvant and injected with the first two of five immunizations to generate T-cell responses in Fischer rats. Immunizing peptide (250 μg) with no repeated administration of OM-CSF. The immunizations were administered on one day only and antigen was applied to rat ear and responses as-assessed at 48 hours. Ear swelling of experimental compared with control ear was measured. Results are depicted as the mean and standard deviation of measurements taken from each experimental group.

20 days with GM-CSF or CFA/IFA for three inoculations. GM-CSF, id, was as effective as CFA as an adjuvant for eliciting rat neu protein specific DTH responses (Fig 9B). Thus, GM-CSF is an effective adjuvant for both protein and peptide based vaccines.

**DISCUSSION**

GM-CSF has many characteristics that would make it an excellent potential vaccine adjuvant. One mechanism by which GM-CSF is hypothesized to augment an immune response is its function as a growth and differentiation factor for epidermal Langerhan’s cells, a class of dendritic cell. These cells are potent APC and thought to be the operative cell type in the initiation of T cell antigen interaction. GM-CSF has been shown to stimulate growth of other APC including blood born DC and macrophages. DC, themselves, have been used as vaccine adjuvants and are capable of inducing significant immune responses when injected along with antigen. Using local injection of GM-CSF to similarly expand the number of APC would be expected to enhance vaccine immunogenicity. Another benefit to the use of GM-CSF as a vaccine adjuvant is its ease of use in humans. GM-CSF has been used extensively as a hematopoietic growth factor in patients undergoing chemotherapy, and repeated administration of the cytokine is generally well tolerated.

Finally, cytokines as adjuvants may influence the character of the immune response generated by influencing the subset of CD4+ helper cell activated, TH1 versus TH2.

Initial investigations, presented here, concentrated on defining the most appropriate method and route of GM-CSF administration for use as an adjuvant. GM-CSF has been described not only as a growth and differentiation factor for epidermal Langerhan’s cells, but also as a chemoattractant for these APCs. It was unknown how long a local injection site would need to be “conditioned” with GM-CSF to recruit and/or grow APC. It is also unknown how long GM-CSF would have to be administered to enhance the efficacy of the recruited cells. A study by Kaplan et al described evaluating Langerhan’s cell recruitment after GM-CSF administration in patients with leprosy. Investigators evaluated the effect of GM-CSF injected into leprosy lesions either id or sq daily for 10 days. They noted recognizable Langerhan’s cells present in the dermis at 48 hours and reaching peak numbers at 4 days when the cytokine was administered id. No Langerhan’s cell infiltration was noted when the cytokine was administered sq. This observation led us to study the effect of GM-CSF administration over a 5-day period. The current studies examined both sq and id routes of administration. Although sq administration did not show increased Langerhan’s recruitment in studies by Kaplan et al, it was a very effective route of immunization with GM-CSF transfected tumors. The sq route has the theoretical advantage of acting as a depot site for the cytokine, thus allowing a longer half-life in the vicinity of antigen deposition. The id route has the advantage of delivering a DC growth factor directly to the skin compartment where DC reside. Once DC are exposed to antigen and activated, they migrate to regional lymph nodes to activate T cells. Using class II expressing cells in lymph nodes draining the injection site as a measure of APC, we noted an effect on these cells in both id and sq immunized groups as compared with controls; however, the kinetics of that effect varied depending on the route of administration used. Sq administration resulted in either an early influx of “APC” into regional lymph nodes or upregulation of class II on cells present in regional lymph nodes with marked differences occurring between the experimental and control groups within the first three injections and then returning to baseline. Id administration resulted in a later effect peaking at the fourth and fifth injections, similar to the results Kaplan et al demonstrated in humans. We evaluated
whether this difference in class II expressing cells in draining lymph nodes translated into functional differences in antigen presentation between the two routes of administration.

Functional experiments evaluating GM-CSF as an adjuvant in a foreign antigen (tt) system were initiated administering antigen late in the cytokine regimen. This time course was tested first based on the assumption that the site for induction of immunity might need to be “conditioned” by inducing the differentiation and growth of APC. The observation that id GM-CSF administration resulted in later changes in class II expressing cells “APC,” was supported by that assumption. Although tt specific antibody responses were generated in both id and sq immunized groups, responses were more consistent in the id immunized animals. This data implies that the changes in class II expressing cells observed with GM-CSF administration did have important functional effects. To test the necessity of site “conditioning” with GM-CSF, we next evaluated the co-administration of GM-CSF and tt early in the cytokine cycle, injecting antigen with the first two of five inoculations of GM-CSF or PBS. Early antigen administration by both the id and sq routes was effective. The id route of administration appeared, again, to be more effective than sq at eliciting tt specific antibody responses. The upregulation of expression of class II MHC on DC has been associated with their maturation and the ability to augment a mixed lymphocyte reaction, but not necessarily with antigen presentation. The efficacy of the id route in early antigen administration may represent loading of antigen on immature DC and subsequent presentation. The continued efficacy later in the cycle may reflect continuing recruitment of immature APC to the site of antigen deposition.

The adjuvant properties of GM-CSF were compared with those of two “standard” adjuvants, CFA and Alum. In addition, vaccination classically involves the administration of the antigen with the adjuvant as a one time dose. Repeated administration of adjuvant after the introduction of the immunizing antigen is not the norm. Experiments were designed not only to compare the use of GM-CSF with other adjuvants, but to tailor the immunizing regimen to one more commonly used in human immunizations: single dose of antigen in adjuvant. GM-CSF was as effective as CFA or Alum in generating DTH response to tt. GM-CSF id was effective in eliciting antibody immunity to titers similar to those seen with the standard adjuvants. Studies are currently underway optimizing the dose of cytokine needed to stimulate an effective immune response.

There are many adjuvants effective in eliciting antibody and T-cell responses to foreign proteins. A more critical issue for developing vaccine therapy for human malignancy is whether adjuvants can elicit T-cell responses to proteins or peptides derived from self tumor antigens. The recent identification of specific tumor antigens has renewed interest in the formulation of a new generation of antigen-directed cancer vaccines. As more of these antigens are elucidated, it becomes clear that a growing number of putative tumor antigens are self proteins. Investigations in our laboratory involve the development of a rat system for evaluating immunity to rat neu as a model for studying immunity to human HER-2/neu. Studies have identified several peptide epitopes, derived from the normal rat neu protein sequence, capable of eliciting T-cell responses in rats when immunized with CFA. Although peptides are generally considered weak immunogens, and these peptides were derived from a self protein, cell mediated immune responses to these peptides were generated by priming with intradermal GM-CSF as a vaccine adjuvant. We presumed even greater responses would ensue following boosting experiments. Using a multiple peptide vaccine known to generate rat neu specific responses in the rat, and CFA as an adjuvant, we administered an initial immunization and two boosting immunizations to the animals. GM-CSF and the peptide based vaccine administered id was as effective as CFA and the peptide vaccine administered sq in generating cell mediated immunity.

The current study identifies GM-CSF as an excellent adjuvant for the elicitation of immunity to both foreign proteins and tumor antigen derived peptides. The cytokine is able to aid in the generation of both antibody and cell mediated immune responses. Studies shown here show the effectiveness of GM-CSF in the initiation of an immune response, without cytokine gene transfection or depot formulation for prolonged exposures at the site of antigen deposition. In addition, GM-CSF compares favorably with other standard adjuvants. The recent observations of an immune response directed against GM-CSF itself, when administered at higher concentrations over time, theoretically may make the intermittent use of standard doses of GM-CSF, as in the current studies, the most effective way to use the cytokine to enhance immune responses. GM-CSF has been used extensively in humans and has shown minimal toxicity even after repeated injections. The use of GM-CSF in humans, as an adjuvant for peptide-based vaccines, may allow the realization of an effective and long-lived immune response to immunogenic epitopes.

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Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines

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