# EGF BASED CANCER VACCINE

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## Introduction

The interaction between epidermal growth factor (EGF) and its receptor (EGF-R) unchains important mechanisms of cellular growth, related with the development of some tumors. Several evidences point to the relationship between the EGF/EGF-R system and human cancer (1-3) making this system a tremendously attractive target for cancer immunotherapy.

Several approaches to cancer treatment has been based in EGF-R targeting, mainly passive immunotherapy with monoclonal antibodies that recognize EGF-R (4, 5). However, these approaches have the drawbacks of passive administration of a foreing protein: unconvenient biodistribution, short half-lifes *in vivo* and allergic reactions.

In this work we propose an alternative way of cancer treatment based in an active immunotherapy with EGF, to induce the production of specific anti-EGF antibodies that may inhibit the EGF/EGF-R interaction.

First, we demonstrated that it is possible to induce the production of antibodies against self EGF, and then we demonstrated that these antibodies alter the biodistribution of injected radioactive EGF and that mice with antibody titer against self EGF have better survival than controls when trasplanted with Ehrlich Ascites Tumor cells.

## Material and Methods

Balb/c mice were immunized either with 50 μg of murine EGF (mu-EGF) linked to cholera toxin B chain (carrier protein), or with 50 μg of human EGF (hu-EGF), by subcutaneous injections on days 0, 7, 14 and 21. Specific antibody titers were measured by ELISA.

The kinetics of antibody response was studied by the measurement of the serum antibody concentration after a single immunization with hu-EGF. When antibody titers decreased mice were re-immunized and the antibody response measured.

Non human primates (IRUS monkeys) were immunized with 500 μg of hu-EGF linked to tetanic toxoid in Freud's adjuvant, subcutaneously, in weeks 1, 2, 3, 4 and 6.

Green monkeys were immunized with 500 μg of hu-EGF linked to tetanic toxoid or linked to P64K *Neisseria meningitides* recombinant protein in Aluminium Hydroxide as adjuvant, subcutaneosly, in weeks 1 and 3.

For biodistribution studies, immunized and non-immunized mice were injected with 125I-EGF and then, in different time intervals, blood samples were taken and mice were sacrificed. Liver, kidney and lung were immediately excised and weighed, and the radioactivity counted.

Biodistribution experiments were repeated in immunized and non immunized tumor bearing mice. In this case, ascites were also taken at various time intervals.

For the assessment of the effect of immunization on survival, groups of mice with and without antibody titers against self-EGF were trasplanted with Ehrlich Ascites Tumor cells, and observed for survival times. Analisis of survival data was made using Wilcoxon and Mantel Haenszel statistical tests.

Transaminases and alcaline phosphatase were measured in sera from immunized and non-immunized mice. Also histological studies were developed in samples of lung, salivary glands, stomach and small and large intestine.

## Results and Discussion

The possibility of inducing the immune system to recognize self EGF was studied in mice and monkeys.

Mice produced antibody titers against mu-EGF up to 1:500 sera dilution when immunized with mu-EGF linked to a carrier protein.

No antibody titers were measured in animals injected only with mu-EGF not linked to a carrier protein. Immunization of mice with unlinked hu-EGF produced antibodies against both, the human and the murine EGF, this result demonstrated induction of immunity against shared epitopes. The response was always of IgG isotype.

When mice were re-immunized after decrease in antibody titers after a single dose immunization, antibody response showed immunological memory.

IRUS monkeys immunized with hu-EGF linked to tetanic toxoid in Freund's adjuvant develòped a long lasting antibody response with titer up to 1:200 000 sera dilution.

Green monkeys immunized with 2 doses of hu-EGF linked to different carrier proteins developed antibody titers up to 1:20 000 sera dilution. These titers decreased in the 2nd month after the last immunization. Monkeys immunized with hu-EGF without carrier protein did not develop antibodoy titers.

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*In vivo* experiments showed that EGF immunization modified biodistribution of injected 125I-EGF. Mice with titers of anti-EGF antibodies accumulated more EGF in liver and less EGF in kidneys as compared with non-immunized, that could indicate a different way of elimination of immunocomplexes between EGF and anti-EGF antibodies. It was also shown a lesser content of EGF in ascites of immunized mice. These results point to the *in vivo* "EGF deprivation" by specific autoantibodies.

The effect of anti-EGF antibodies on tumor development was studied in mice trasplanted with Ehrlich Ascites Tumor (EAT), a trasplantable tumor with content of EGF-R. In 20 consecutive and independent experiments we observed increased survival times in EGF immunized mice trasplanted with EAT as compared with mice treated only with adjuvant.

The values of increase life span (ILS) in these experiments were in a range bewtween 10 % and 99 %. In the 85 % of the experiments the differences in survival times between immunized mice and controls were statistical significative, according to Wilcoxon and Mantel Haenszel tests.

We did not observe any effect over functional hepatic parameters of immunized mice compared with controls. Neither was observed any histological damage. In 19 monkeys immunized with hu-EGF coupled to different carrier proteins and using different immunization protocols we did not observe any sign of toxicity of the treatment.

These results support the idea of an "EGF-vaccine" for the treatment of EGF-dependent malignant tumors.

# THE INHIBITORY EFFECT OF TUMOR-SHED GANGLIOSIDES ON THE PRODUCTION OF IL-1BETA BY MONOCYTES AS WELL AS ON THE PROLIFERATIVE ACTIVITY OF THIS LYMPHOKINE ON THYMOCYTES DEPENDS ON THE STRUCTURES OF BOTH OLIGOSACCHARIDE AND CERAMIDE MOIETIES OF THE GANGLIOSIDES

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Gangliosides are sialic acid-containing glycosphingolipids made of an oligosaccharide bound to a ceramide moiety. These molecules are highly exposed at the cell surface of tumor cells and one of the mechanisms by which the tumors escape the immune system is thought to involve shedding of gangliosides from the cell surface into the extracellular medium and the subsequent uptake of shed gangliosides by lymphocytes. Shedding is an active phenomenon closely related to the rate of proliferation and the amount of gangliosides released by tumor cells is equal after three to four days to the total cellular ganglioside content. At concentrations frequently found in the sera of tumor-bearing patients, the *in vitro* uptake of tumor-associated gangliosides during three days by monocytes leads to the insertion of these gangliosides in their plasma membrane. Such an enrichment of gangliosides results in a dramatic decrease in the production of IL-1β upon activation of monocytes by LPS. A study was carried out using several gangliosides known to be major components of various tumors and their effects were widely different. The production of IL-1β by LPS-activated monocytes was highly sensitive to gangliosides and GM2 had the most inhibitory effect whereas it had a very weak influence on the proliferative activity of IL-1β on thymocytes. At the opposite, GM3 showed a much more potent inhibition on the activity of IL-1β than on its production.

The potency of gangliosides as inhibitors of IL-1β production was in the decreasing order: GM2 > GD1a > 9-OAcGD3 > GD2 >> GT1b > GM3 > GD3 > GD1b. The study of these gangliosides as inhibitors of IL-1β dependent thymoproliferation showed quite different effects and the order of potency was the following: 9-OAcGD3 > GM3 > GD2 > GD1b > GT1b > GD3 > GD1a > GM2. These results suggest that the extent of immunomodulation by tumor-shed gangliosides depends greatly on the ganglioside pattern of the tumors.