

Antibody-mediated immune response to EGF delivered by hydroxyapatite nanoparticles: a strategy for long-term cancer vaccination

Yerandy Hechavarria Luna^{a1,2,3} and María del Carmen Luzardo Lorenzo^{1,3}

¹Centro de Estudios de Proteínas (CEP), Facultad de Biología, Universidad de La Habana, La Habana, Cuba.

²Laboratório de Bioinsumos – BIOLIDER, Novagrolider, Angola.

³Laboratorio de NanoCancer, Centro de Inmunología Molecular (CIM), La Habana, Cuba.

CIMAvax-EGF controls circulating epidermal growth factor (EGF) through a vaccine-induced antibody response, but prolonged administration of mineral-oil adjuvants can cause tissue damage at the injection site and in adjacent areas. We evaluated the humoral immunogenicity and maintenance of anti-EGF antibodies elicited by hydroxyapatite nanoparticle (HANp) systems, showing sustained IgG responses, predominantly IgG1/Th2, and preservation of antibody levels after replacing CIMAvax-EGF maintenance doses with HANp-rhEGF-rP64k.

Brief research communication based on previously published experimental work [1].

Why the immune response matters

CIMAvax-EGF is a therapeutic cancer vaccine designed to disrupt the EGF–EGFR axis. Its mechanism is antibody-mediated: anti-EGF antibodies sequester circulating EGF and reduce its interaction with EGFR, thereby limiting proliferative and pro-survival signaling [1, 2]. The clinical usefulness of this approach depends not only on inducing antibodies, but on maintaining them during prolonged treatment. The original study therefore asked an immunological question with direct translational relevance: can a biodegradable calcium-phosphate platform reproduce and sustain the humoral response generated by the current formulation?

Immunological evaluation

Female C57BL/6 mice (8–12 weeks; n=5/group) received four intramuscular immunizations at 14-day intervals (days 0, 14, 28 and 42). Three HANp formulations—HANp-rhEGF-rP64k, HANp-MC and HANp-IP—contained 158 μ g HANp and 63 μ g antigen per dose; CIMAvax-EGF (63 μ g rhEGF-rP64k in Montanide ISA 51 VG) was the comparator. Sera were collected before immunization and on days 35, 56 and 104. Anti-rhEGF total IgG and IgG1, IgG2b and IgG2c subclasses were quantified by ELISA. The Th1/Th2 balance was represented by (IgG2b+IgG2c)/IgG1. A separate priming/boosting experiment used one or two CIMAvax-EGF induction doses followed by HANp-rhEGF-rP64k, with a fifth maintenance dose on day 77; sera were evaluated through day 91 [1].

What the immune system did

All three HANp formulations induced detectable anti-EGF IgG after vaccination, with titers of approximately 1:5,000–1:10,000 across the analyzed

time points. HANp-MC and HANp-rhEGF-rP64k produced comparable responses, whereas HANp-IP showed lower titers at several early time points but remained stable through day 104. Importantly, the nanoparticle responses were lower than those elicited by CIMAvax-EGF under the tested dose conditions, identifying formulation or dose optimization as the logical next step rather than evidence of immunological failure [1].

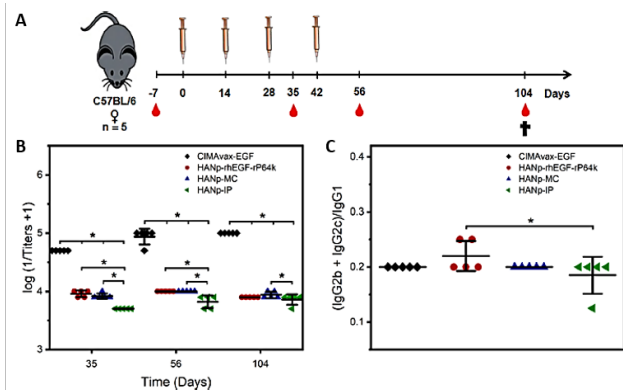


Figure 1. Anti-EGF humoral response induced by hydroxyapatite nanoparticle systems. (A) Experimental immunization schedule. (B) Total anti-EGF IgG titers induced by CIMAvax-EGF and HANp-based formulations. (C) Th1/Th2-associated antibody subclass ratio calculated as (IgG2b + IgG2c)/IgG1 on day 56. Data and statistical analysis are reproduced from the previously published study [1].

The antibody subclass profile provided the most informative immunological finding. For all formulations, the (IgG2b+IgG2c)/IgG1 ratio remained below 1, with no significant difference from CIMAvax-EGF. Thus, the response was predominantly IgG1 and consistent with a Th2-biased humoral phenotype. This is mechanistically coherent with an EGF-depleting vaccine whose immediate effector function is antibody se-

questration of a soluble self-protein rather than direct cytotoxic recognition of tumor cells [1, 3]. Although IgG2 subclasses were detectable, the study did not demonstrate a statistically significant shift toward a Th1 phenotype; therefore, claims of enhanced cellular immunity would go beyond the available data.

The maintenance experiment was particularly relevant. Mice primed with one or two CIMAvax-EGF doses and subsequently boosted with HANp-rhEGF-rP64k maintained total IgG as well as IgG1, IgG2b and IgG2c titers without significant differences from mice receiving CIMAvax-EGF throughout the schedule. Antibody levels were maintained over the 91-day observation period. These findings support the concept of separating induction from maintenance: a potent oil-adjuvanted formulation can establish the response, whereas a biodegradable nanoparticulate formulation can potentially sustain it [1, 4].

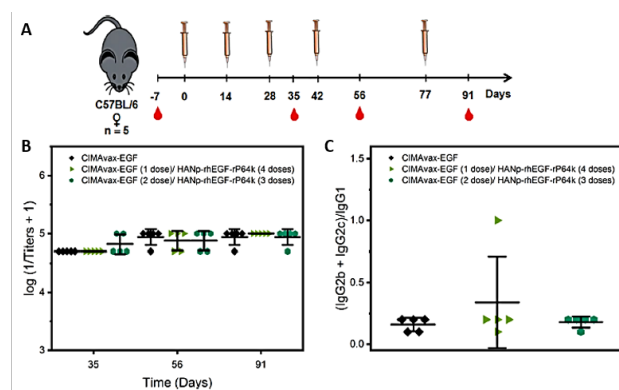


Figure 2. Maintenance of the anti-EGF immune response after switching from CIMAvax-EGF to HANp-rhEGF-rP64k. (A) Priming and boosting experimental design. (B) Total anti-EGF IgG titers during the study. (C) IgG subclass ratio on day 56. HANp-rhEGF-rP64k maintained antibody responses after one or two induction doses with CIMAvax-EGF [1].

An immune response with reduced local tissue damage

The immunological advantage is meaningful only if repeated administration is locally tolerable. Histological examination after 104 days showed lesions in all five animals in the CIMAvax-EGF group, compared with one of five animals in the HANp-rhEGF-rP64k group, one of five in the HANp-MC group, and three of five in the HANp-IP group. The corresponding mean in-

jury scores were 5.0, 0.2, 0.2 and 0.8, respectively. The marked reduction in tissue damage, together with preserved anti-EGF antibody production, strengthens the rationale for HANp as a maintenance platform [1].

The immunological message

The central result is not simply that hydroxyapatite nanoparticles are immunogenic. Rather, they can generate a sustained anti-EGF humoral response and, in a priming/boosting setting, maintain antibody levels previously established with CIMAvax-EGF. This suggests a clinically attractive vaccine architecture: strong immune induction followed by repeated maintenance with a biodegradable adjuvant. The next immunological questions are dose optimization, durability of the maintenance response beyond the 91-day observation period, functional neutralization of EGF, and whether selected nanoparticle designs can increase the cellular component without compromising the antibody-mediated mechanism.

Notes

a. Email: yerandyhechavarrialuna@gmail.com.

References

- [1] González-Martínez, D. A., et al. Hydroxyapatite Nanoparticles as a Potential Long-Term Treatment of Cancer of Epithelial Origin. *ACS Applied Nano Materials* 5, 6159–6170 (2022). <https://doi.org/10.1021/acsanm.2c00021>.
- [2] Saavedra, D., & Crombet, T. CIMAvax-EGF: A New Therapeutic Vaccine for Advanced Non-Small Cell Lung Cancer Patients. *Frontiers in Immunology* 8, 269 (2017). <https://doi.org/10.3389/fimmu.2017.00269>.
- [3] Lambert, S. L., Okada, C. Y., & Levy, R. TCR Vaccines against a Murine T Cell Lymphoma: A Primary Role for Antibodies of the IgG2c Class in Tumor Protection. *The Journal of Immunology* 172, 929–936 (2004). <https://doi.org/10.4049/jimmunol.172.2.929>.
- [4] Rodriguez, P. C., et al. Priming and Boosting Determinants on the Antibody Response to an Epidermal Growth Factor-Based Cancer Vaccine. *Vaccine* 26, 4647–4654 (2008). <https://doi.org/10.1016/j.vaccine.2008.07.003>.