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Gold Nanoparticles Stimulate the Maturation and Activation of Mouse Bone Marrow Dendritic Cells

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***Abstract*— Nanomedicine is an interdisciplinary science that links nanotechnology, nanoscience and nanoengineering to the field of health and the use of nanoscale tools, in this case, nanoparticles have great therapeutic potential against target cells such as dendritic cells (DC). DCs are highly motile cells whose main function is antigen presentation; they are mediators between innate and adaptive immunity. In this investigation, DCs were differentiated and matured with granulocyte-macrophage colony stimulating factor (GM-CSF), 25 ug/mL and spherical gold nanoparticles (AuNPs) of approximately 30 nm at concentrations of 5 to 200 ug/mL. DCs were obtained from the bone marrow of adult mice; their characterization was performed by means of RT-PCR, scanning electron microscopy (SEM) and confocal to know their morphology and the location of the nanoparticles inside. The bone marrow DCs (bmDCs) possibly differentiated into cCD1 dendritic cells, their mature morphological status was determined by the presence of co-stimulatory factors (CD40, CD86 and CD11c), in addition to MHC II and CIITA when in contact with AuNPs, the results were confirmed by SEM observation due to their star-like shape. In terms of the location of the AuNPs they would be clustered and outside the nucleus. The discovery of nanoparticles that do not cause significant cytotoxicity, activate dendritic cells, and produce cytokines, causing phenotypic changes in them, enhancing, and modulating the adaptive immune response or generating a targeted immunotherapy in the presence of different pathogens in the organism.**

***Keywords*—Nanomedicine; dendritic cells; gold nanoparticles.**

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I. INTRODUCTION

The immune system is made up of various organs, tissues, and cells, including antigen-presenting cells (APCs) such as macrophages and dendritic cells. APCs engulf foreign particles or microorganisms and stimulate the production of lymphocytes and other immune cells by releasing chemical mediators or cytokines, which are responsible for initiating the adaptive immune response [1]. According to Hilligan [2], the immune system for antigen presentation, lymphocyte activation, and the restricted migration and low production of dendritic cells limits a potential defense response. To benefit from a large-scale immunotherapy, novel approaches should be investigated to target dendritic cells antigens, drugs, and immunostimulatory agents.

The Dendritic Cells (DCs) constitute approximately 1% of animal white blood cells. They have numerous membrane projections, are highly motile, and specialize in antigen presentation to immature T lymphocytes due to their ability to optimally capture, process, and expose pathogens to generate a specific immune response [3]. They are found in areas exposed to foreign infectious agents or tissue damage where they capture antigens and migrate to the lymph nodes to process and present antigens [4]. They, and, when activated, [5]. Dendritic cells are the first to reach the site of infection and are considered mediators between the immune responses as they are attracted and activated by elements of the first innate defense mechanism and allow the sensitization of lymphocytes of the adaptive response [6]. DCs are characterized by their ability to express a high concentration of class II major histocompatibility complex (MHC-II), the absence of lineage markers. In addition, they present common adhesion molecules for monocytes and macrophages and co-stimulatory molecules. [7], [8].

DCs originate from hematopoietic precursors, with the influence of FLT3 and GM-CSF [6]. Two types of progenitors are obtained: lymphoid and myeloid cells [7]. These progenitors are phenotypically and functionally different dendritic cells. They circulate in the peripheral blood and are

distinguished from each other by the expression of CD11c and CD123.

DCs are in three functional states: immature, semi-mature, and mature; they differentiate themselves by their ability to interact with virgin cells. Being immature, they are tolerogenic and essential to prevent responses against healthy tissues, unlike mature ones that are immunostimulant [9]. Most dendritic cells *in vivo* are in an immature and inactive state. Consequently, they can be converted to a mature and active state with different phenotypic and functional characteristics by exposure of these cells to different stimulatory ligands [10].

Nanoparticles possess greater reactivity and stability when they range from 1 to 100 nm in size because they have unique physical, chemical, and biological properties at the nanoscale [11]. Nanoparticles have been introduced in different areas of interest in biology and medicine, from diagnostics to disease therapy.

One of the main characteristics of nanoparticles is their ability to induce immune responses, such as antibody production and positive regulation of cytokines. Their physical and chemical structure grants them different effects that can be applied in future applications in biomedicine [12]. Dendritic cells can interact with nanoparticles as they are foreign to the body. The effectiveness of retention depends on the shape and mainly on size of the nanoparticles [13].

Gold nanoparticles (AuNPs) are easy to prepare, highly modifiable, and biocompatible because gold is considered biologically non-reactive, so they are recommended for *in vivo* applications [16]. However, no FDA-approved clinical trials have been registered with nanoparticles, but their consumption in a colloidal state has been accepted [17].

AuNPs have a high molecular density on their surface and unique optical properties, making them a research target as an immunotherapy agent. This type of nanoparticle has been studied, used for immunotherapies, and employed in vaccine development. Several factors have to be considered to develop their immunomodulatory capacities, such as the selection of cells or organs, cellular absorption capacity, and release kinetics [18], [14].

The toxicity of AuNPs is related to shape, size, and surface charge, as they can directly affect recognition and acceptance by dendritic cells. However, AuNPs range in size from 14 to 100 nm, which can easily enter mammalian cells without causing cytotoxicity. Due to their small size, they tend to accumulate mainly in immune cells and passively in solid tumors [15].

AuNps could be an alternative method to conventional chemotherapy with little to no aggressive side effects. They also can be conjugated to specific drugs and used as a vehicle; for the latter, there are already several applications in preclinical and clinical trials [15]. In DCs, gold nanoparticles have been involved in immunomodulation by helping them to improve antigen uptake, an intracellular targeting. AuNps-maturated DCs enhance antigen absorption and initiation of adaptive immunity since they make up an effective targeting [19].

According to Flórez-Grau et al. [20], most clinical trials seek to exploit their prevalence through *in vivo* and *ex vivo* manipulation of dendritic cell precursors such as MoDC to elicit T-cell immunity; one of their main applications is tolerogenic dendritic cells as a vaccine base against different diseases.

Nanotechnological and bioengineering developments have improved the safety and efficacy of immunotherapy. This way, their action focuses on particular cells and locations [21]. Nanoparticles are processed and recognized by immune cells, including macrophages, where their interaction produces IL-8, a chemokine that attracts and activates neutrophils, enhancing the innate immune response. In addition, increased T-cell adhesion and stimulation enhance adaptive immunity [22].

In accordance with current FDA regulations [23], before *in vivo* tests, the stability and biocompatibility of nanomaterials should be tested in contact with proteins in the culture medium, and blood must be taken into account, considering that multiple interactions form a crown of proteins. The FDA-approved nanoparticles are liposomal, polymeric, micellar, metallic, and protein particles [24].

This research aims to address and verify whether spherical AuNPs, 30 nm in size, negative surface charge, and citrate as a stabilizer differentiate and mature bone marrow-derived dendritic cells to enhance adaptive immune response. The results will be used to generate targeted immunotherapy against different pathogens present in the body or modulate the immune response in immunosuppressed people in further studies.

II. Materials And Methods

The research project was carried out in the Immunology - Virology Laboratory and Nanomaterials Characterization Laboratory, belonging to the Department of Life Sciences and Agriculture of the Universidad de las Fuerzas Armadas ESPE, located in Sangolquí, Av. El Progreso s / n, Cantón Rumiñahui, Pichincha province, Ecuador.

A. Animals

We chose female and male BALB / c mice between 9 and 24 weeks of age. For each test, we used one to three mice. Complying with the 3R's, we euthanized each individual by cervical dislocation and obtained 4 bones (2 femurs and 2 tibias).

B. Obtaining BMDCs.

Once disinfected, we centrifuged, at 5000 RPM in 150 uL of RPMI media and extracted the marrow containing bone marrow dendritic cells (bmDCs). We then performed a red blood cell lysis with 500 uL of lysis buffer and constant homogenization for a minute. To stop the reaction, we added 500 uL of RPMI 1640 followed by centrifugation at 5000 RPM for 2 minutes. The cell pellet was resuspended in 1 mL of medium. The cells were counted in a Neubauer chamber and seeded at approximately 5 x10^6 cells in each culture flask with 3 mL of RPMI 1640 medium supplemented with 1% L-glutamine, 10% fetal calf serum, 0.1% 50 mM β-mercaptoethanol, 1% penicillin/streptomycin/amphotericin, 1% non-essential MEM and 1 % MEM pyruvate; cells were incubated at 37 ° C and a humidified atmosphere of 5% CO_2.

C. Synthesis of Gold Nanoparticles

The gold nanoparticles used in this research were donated by the Immunology and Virology Laboratory, Center for

Nanoscience and Nanotechnology, Universidad de las Fuerzas Armadas – ESPE.

D. Stimulation

After 24 hours of culture, we removed half of the medium and added the same amount increasing 20 ng / mL of GM-CSF as a positive differentiation control, gold nanoparticles of approximately 30 nm as treatment at 5, 12.5, 25, 62.5, 100, 112.5 and 200 ug / mL concentration and pure medium as a negative control. Cells were changed medium as required, taking care of each treatment and control, maintaining culture conditions for 12 days with periodic observation under an inverted microscope. Twelve days prior to lifting the assay, the cells cultured for positive control were exposed to *Escherichia coli*, to achieve maturation of the differentiated dendritic cells.

E. RNA Extraction, cDNA Synthesis, and End Point PCR.

The RNA extraction was carried out according to the indications of the SV Total RNA Isolation System kit. The obtained RNA was quantified in the NanoDrop 2000 equipment (Thermo Scientific). The ideal quality to perform cDNA synthesis from RNA was found to be approximately 2.0 for the 260/280 ratio and between 1.8 to 2.2 for the 260/230 ratio [24]. To obtain cDNA, we worked with the High-Capacity cDNA Reverse Transcription kit. With the obtained cDNA, an end-point PCR was carried out to detect surface maturation markers such as CD40, CD11c, CLEC9A, CIITA, and CD86. The PCR product was developed on a 1.5% agarose gel at 90 V for 1 hour. The data was analyzed in the Image Lab program of the Bio-Rad ChemiDoc MP equipment.

F. Scanning Electron Microscope (SEM).

SEM performed the observation of dendritic cells. We cultured the cells on coverslips and fixed them with 4% paraformaldehyde and RPMI 1640 (v/v) for 5 minutes. We then removed the mixture and added 600 uL of 2% paraformaldehyde in 1X PBS for 40 minutes at room temperature. We washed the cells with 500 uL of distilled water, and dehydrated with an ascending sequence of ethanol with 15 minutes of incubation per ethanol concentration. The absolute ethanol was evaporated for 1 hour at 37 °C. Finally, the samples were coated for 90 seconds with gold and observed under the Tescan MIRA 3 microscope at 10.0 kV.

G. Confocal Microscope Observation

To determine the location of the AuNPs within the cells, we stained the cytoplasm with 50 uL of Alexa Fluor 50 uM and incubated for an hour at room temperature, taking care of the light, and stained with 4 uL of DAPI for the nuclei in 1000 uL of 1X PBS. The observation was made under the Olympus Fluoview FV1200 confocal microscope with 458 Multi Argon lasers for DAPI whose excitation wavelength is 341 nm and emission wavelength of 452 nm according to the fluorophore instruction manual, and 635 LD for Alexa Fluor 633 with an excitation wavelength of 633 nm. In the case of gold nanoparticles, a virtual fluorescence channel was generated because of their high atomic number (Z = 79) compared to organic matter. The electrons cross the sample and are scattered at angles depending on the Z number, thus generating their fluorescence [25].

H. Statistical Analysis

We used the GraphPad Prism 6 software for statistical analysis, employing Dunnett's statistical tests and one-factor ANOVA with a significance of 0.05 and p-value <0.05 to accept or reject null hypotheses.

III. Results and Discussion

Gold nanoparticles are widely used in biomedicine thanks to their unique physicochemical and optical properties. However, their toxicity is closely related to shape, size, and surface charge, as they can directly affect recognition and acceptance by dendritic cells [12]. Therefore, using spherical gold nanoparticles of chemical synthesis, with citrate as a stabilizer, of approximately 30 nm in diameter, with negative surface charge complies with appropriate factors to avoid cell death [1] and generate differentiation and maturation of bone marrow-derived dendritic cells.

BALB / c mice between 8 to 52 weeks of age showed total cellularity of the marrow of 530 ± 20 million cells for each animal. From 8 to 24 weeks of life, the cell density is constant. Furthermore, approximately 10% of the total hematopoietic stem cells are found in the femurs and 4% in the tibiae [26] when using a marrow wash as an extraction procedure. The amount of viable cells extracted is approximately $2.5x10^7$ to $3.5x10^7$ per animal [5]. These results agree with the cell density of this study, where it was possible to obtain up to $3.05x10^7$ cells in 1 mL of medium per animal. Figure 1 details that the number of cells is in the mentioned ranges. Therefore, the studied animals reached the highest number of viable dendritic cell precursors between 9 and 24 weeks of age.

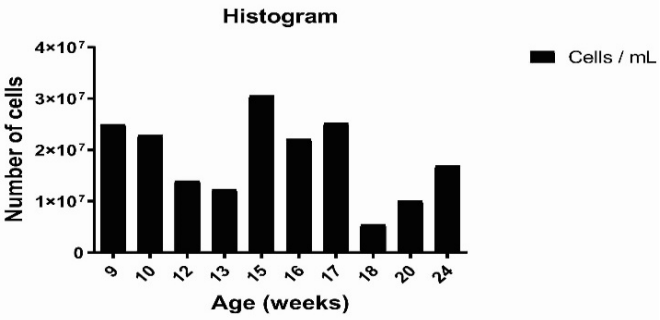

Fig. 1 Mouse age and cell density histogram. Cell densities are between $5.5x10^6$ and $3.05x10^7$ cells/mL for 9-24-week-old BALB / c mice. The average cell density of $1.8x10^7$.

According to Showalter et al. [27], the culture time depends on the use of the dendritic cells. For this reason, they suggest that the cells should be used after ten days of culture; cells with treatment (25 ug / mL) semi-adhered after 12 days of culture present a circular structure with membrane projections similar to a star as seen in figure 2, adhered cells present morphology similar to the positive control (Fig 3.) showing a monolayer formation with high confluence.

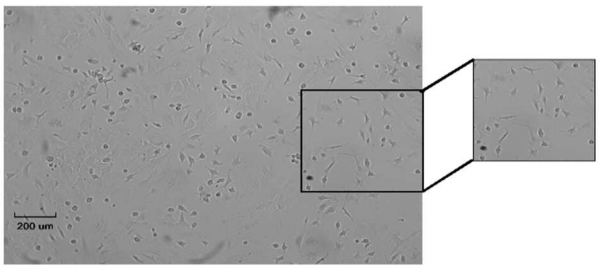

Fig. 2 Brightfield micrograph of dendritic cells with AuNPs (25 ng / mL) isolated from mouse bone marrow. Undifferentiated cells remain circular (red), and differentiated cells (white). Magnification 100X.

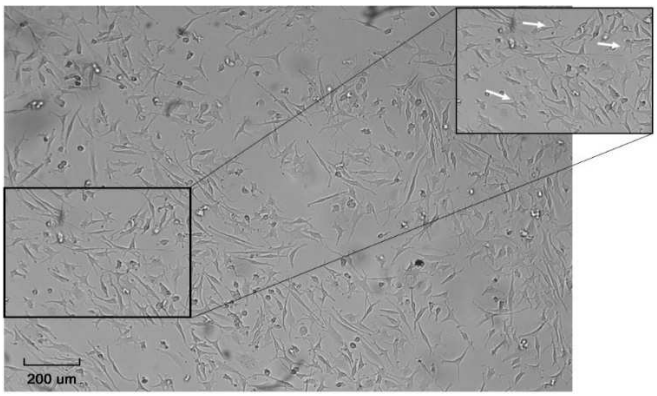

Fig. 3 Brightfield micrograph of dendritic cells stimulated with 20 ng / mL gm-csf as a positive control, isolated from mouse bone marrow. 100X magnification.

After 12 days of culture, 11 with treatments (5, 25, 100, and 200 ug / mL AuNPs), the test ended. We examined the cells to determine the cell viability induced by the different concentrations of gold nanoparticles by means of trypan blue staining. Figure 4 shows the positive control with a cell density of $5.6x10^6$ cells / mL, significantly higher than the treatments and baseline control.

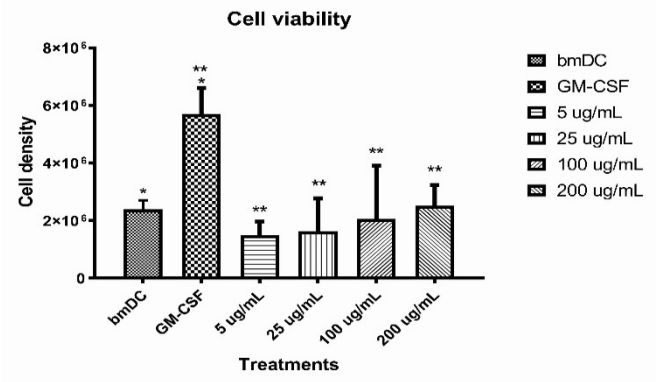

Fig. 4 Graphs of means and standard deviation for cell viability after 11 days of treatment under the different test conditions. *: difference in means between treatments. With a Holm Sidak test, the difference of means was found between the treatments with Np and the baseline control with the positive control, that is, GM-CSF; There is no significant difference between nanoparticle concentrations (p_value> 0.05).

According to Niikura et al. [1], spheres between 20 and 40 nm induce less cytotoxicity, and citrate-coated nanoparticles between 10 and 50 nm did not damage embryonic fibroblasts up to a concentration of 300 mmol/L. Higher concentrations resulted in changes in cell morphology. For capture and transport, the immune system has an affinity for nanomaterials between 50 and 500 nm in size [13]. However, high amounts of free citrate can cause a loss of viability. Likewise, the positive charge depolarizes the membrane. Therefore, an increase in [Ca2+] could be generated, inhibiting cell proliferation [28]. In other words, the cells subjected to treatment would differentiate and mature but not proliferate.

The expression of co-stimulation factors such as CD40, CD11c, CLEC9A, and DC86, and the presence of MHC-II and CIITA, as well as β-actin, constitutive gene, and P53 indicator of apoptosis or cell death were evaluated by end-point PCR, 1.5% agarose gel and densitometric analysis.

β – actin (Fig. 5) is higher for the treatments and positive control compared to the negative control. However, between the AuNPs treatments, no significant differences were evidenced. MHC-II expression is higher in cells exposed to 12.5 ug / mL of AuNPs (Fig. 6) than in control cells. There is a significant difference in means between the AuNPs treatments and the negative control, in the same way when comparing the means of treatments with positive control using Dunnett's multiple comparison test.

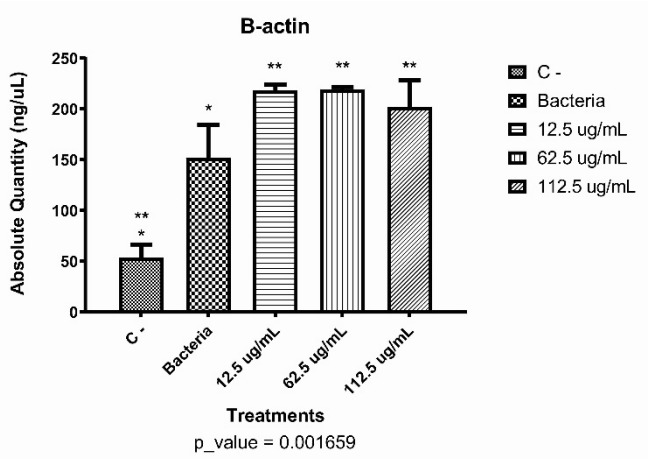

Fig. 5 Bar chart of β-actin expression by densitometric analysis. * = standard deviation, p_value <0.05. C -: Negative control.

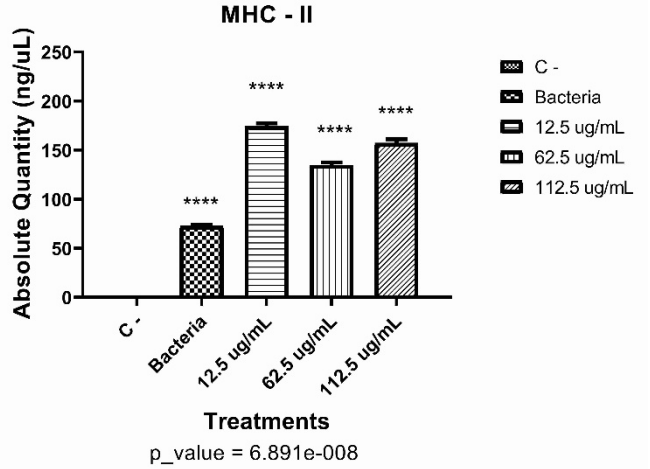

Fig. 6 Bar chart of MHC-II expression bar diagram by densitometric analysis. * = standard deviation, p_value <0.05C -: Negative control.

The transactivating protein of MHC-II, CIITA (Fig. 7), was expressed in greater quantity in the positive control and in the AuNPs treatments with a significance of 0.05 and p-value

<0.05, it is concluded that there is a significant difference between the means of the treatments and the positive control in relation to the negative control, on the other hand, there is no significant difference in means of gene expression between the positive control and the AuNPs treatments.

The gene for the CD40 protein was expressed in greater quantity in the negative control, followed by the 12.5 and 62.5 ug/mL treatments, unlike the positive control and the treatment with the highest concentration. However, there is no significant difference in means between the positive control and the 12.5 and 62.5 ug/mL AuNPs treatments (Fig. 8).

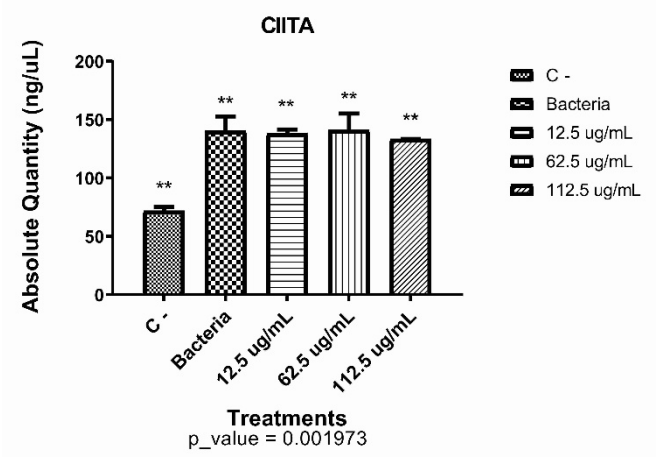

Fig. 7 CIITA expression bar diagram by densitometric analysis. * = standard deviation, p_value <0.05. C -: Negative control.

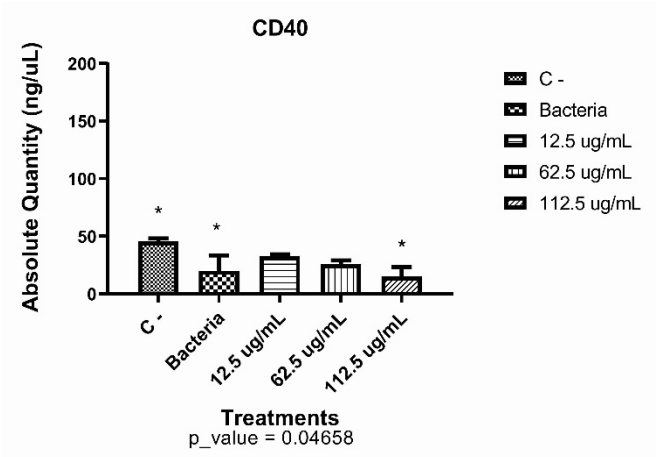

Fig. 8 Bar chart of CD40 expression by densitometric analysis. * = standard deviation, p_value <0.05. C -: Negative control.

Figure 9 shows the expression of Clec9A is significantly higher in the positive control than in other treatments. In addition, there is a significant difference concerning the expression of Clec9A in the negative control, the positive control, and the AuNPs treatments. There is no significant difference when comparing the expression between treatments. The gene for CD86 (Fig. 10) was not expressed in the negative control. No significant difference between the expression of CD86 in the positive control and the nanoparticle treatments was found.

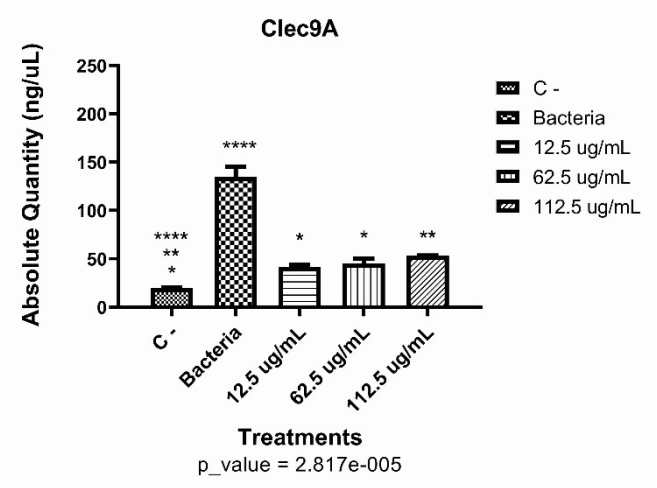

Fig. 9 Bar diagram of expression of Clec9A by densitometric analysis. * = standard deviation, p_value <0.05. C -: Negative control.

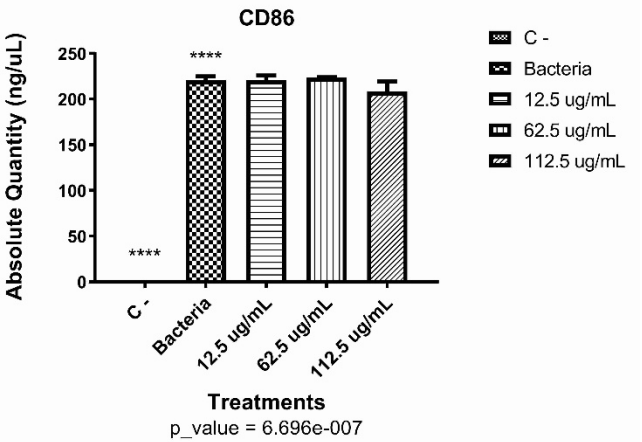

Fig. 10 Bar diagram of CD86 expression by densitometric analysis. * = standard deviation, p-value <0.05. C -: Negative control.

According to the results obtained, all concentrations of nanoparticles (5, 12.5, 25, 62.5, 100, 112.5, and 200 ug / mL) stimulate the differentiation and maturation of dendritic cells. However, when using 5 and 200 ug / mL of AuNPs, the cells showed greater cell death, comparing these results with those of the expression of p53 (Fig. 11) and viability (Fig. 4). The number of cells obtained with 5 ug / mL would indicate that this concentration is toxic for use as a treatment. The ideal concentrations of gold nanoparticles were 12.5, and 25 ug / mL as the expression of co-stimulation and maturation genes is significantly higher.

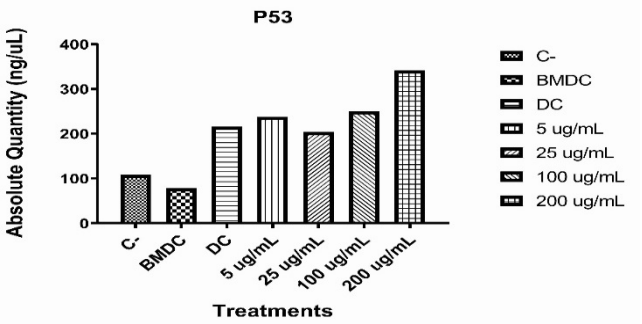

Fig. 11 Bar densitometric diagram analysis of p53 expression in cells derived from mouse bone marrow exposed to different treatments. * = standard deviation, p_value <0.05.

When DCs are exposed to foreign stimuli, they improve their ability to present antigens, causing phenotypic changes; that is, they go from an immature to mature state, accompanied by an increase in the expression of MHC-II and co-stimulatory molecules such as CD40, CD80 and CD86 [29]. After 12 days of stimulation of the BMDCs with GM-CSF and E. coli (LPS), the expression of MHC-II, CD 86, and 95% of CD11c was stimulated in 50% of the cells. The interaction of the 4 co-stimulatory molecules and MHC-II generates a state of complete maturation [30]. In figure 6, the expression of MHCII is higher in the treatments with AuNPs compared to the controls, even in the highest concentrations; CD40 co-factor (Fig. 8) was expressed in greater quantity in cells without external stimuli. However, compared to the positive control, its presence in the treatments is greater or equal; in the expression of CD86 (Fig. 10) there is no significant difference between the treatments and the positive control, CD86 is absent in the negative control. During the maturation of DCs, the expression of MHC-II increases. Similarly, the de novo biosynthesis of the complex's mRNA decreases due to the accelerated reduction of the class II transactivator protein (CIITA). When the cell is immature, isoforms I and III are abundant [31]. The presence of CIITA (Fig. 7) is since, according to Jin and Sprent [30], at 12 days of culture, only 50% of the cells generate conditions of complete maturation.

We used DNGR-1 encoded by Clec9A to determine the type of dendritic cells present in our assay. This type C lectin receptor is restricted to conventional type 1 dendritic cells (cDC1), common progenitors of DC (CDP) and pre-DC, and to a lesser extent, in pDC. However, it does not induce activation characteristics in cDC1, unlike co-stimulatory molecules [32]. Therefore, its presence (Fig. 9) indicates the possible differentiation in cDC1 for the positive control cells and is significantly lower for gold nanoparticle treatments.

Regarding the toxicity of AuNPs, the presence of the p53 protein indicates processes associated with cell life and death, such as regulation and induction of apoptosis when DNA damage is severe, regulation of the stage of senescence, and transport in cells in the later stages of the cell cycle [33]. In this case, P53 is expressed in greater quantity in 5 and 200 ug / mL of gold nanoparticles (Fig. 11). In the other treatments, the expression is similar to the positive control. However, this process mediated by p53 cannot be determined. Therefore, the ideal AuNPs concentrations for DC stimulation to avoid cell damage are 12.5 and 25 ug / mL.

The absorption, internalization, and elimination of nanoparticles by dendritic cells depend mainly on their size and charge [28]. The binding of proteins to the nanoparticles would alter their physical-chemical properties, the AuNPs, mainly, would form a crown of proteins, altering their surface charge, thus facilitating cell uptake through receptor-mediated endocytosis, as well as avoiding their aggregation. 50 nm nanoparticles are more efficiently absorbed; however, they can aggregate within cells [19].

When DCs are in an immature state, they undergo a series of changes after absorption and antigen processing and obtain mature phenotypes and functions, among them morphological changes [5]; when observed through scanning electron microscopy, mononuclear cells derived from bone marrow are circular and small in size [34] (see Fig. 12).

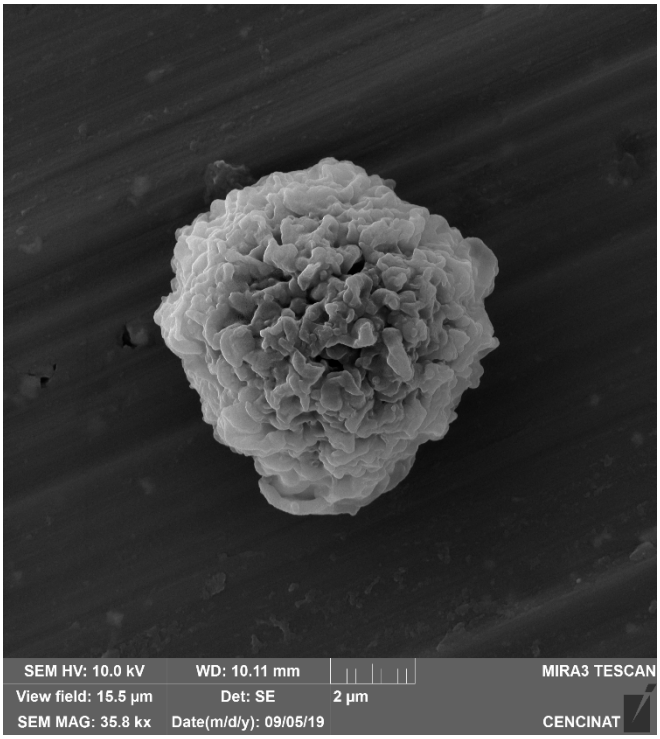

Fig. 12 Immature dendritic cells. Scale: 10.11 mm. Culture time = 12 days. SEM observation at 10.0 kV with SE detector.

On the other hand, mature dendritic cells present a stellate appearance with numerous "dendrite" protrusions of different sizes. They also have a rough surface, being these its typical morphological characteristics. These features coincide with the micrographs obtained in this investigation, as seen in figure 13 and 14.

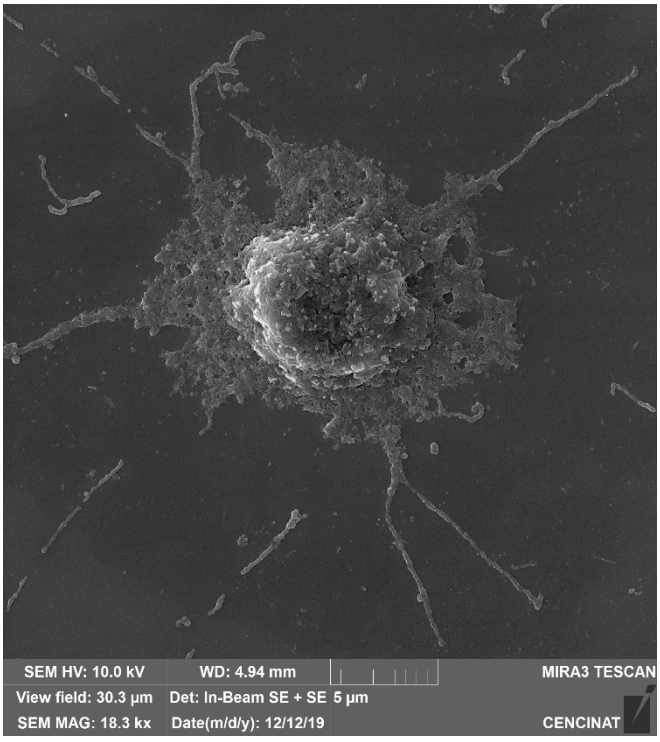

Fig. 13 Mature dendritic cells differentiated with 20 ng / mL of GM-CSF. Scale: 4.94 mm. Culture time = 12 days. SEM observation at 10.0 kV with In-Beam SE + SE detector.

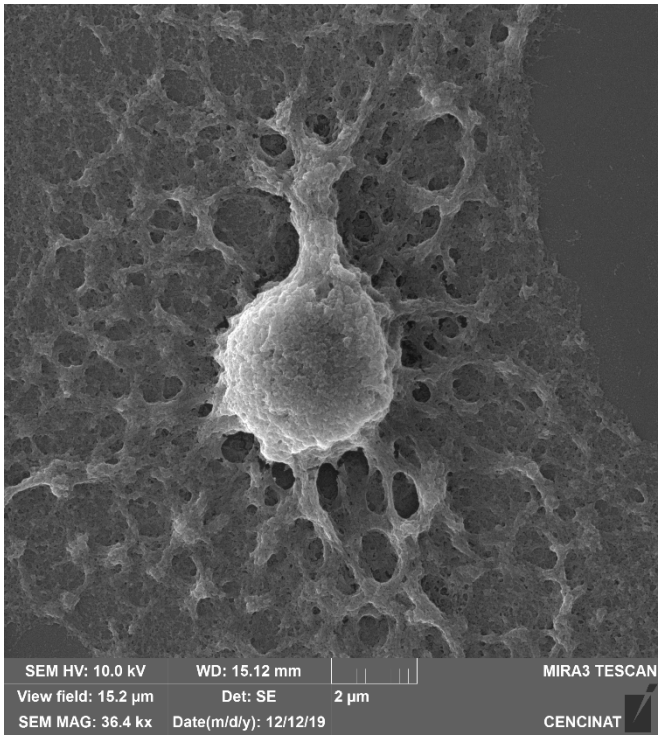

Fig. 14 Mature dendritic cells differentiated with 12.5 ng / mL of gold nanoparticles. Scale: 15.12 mm. Culture time = 12 days. SEM observation at 10.0 kV with SE detector.

Figure 15 shows the presence of green gold nanoparticles in the cytoplasm of red color near the nucleus of blue coloration. When grouped, they differ from the negative control as the observed image lacks green coloring, thus ruling out the presence of nanoparticles.

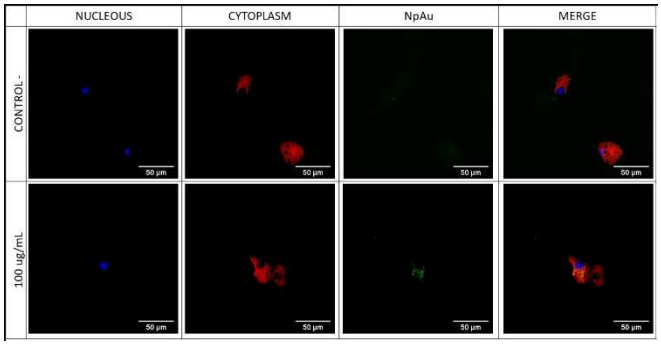

Fig. 15 Confocal microscope micrograph of dendritic cells. Magnification: 600X, core staining: DAPI (blue), cytoplasm staining: Alexa Fluor 633 (red), AuNPs (green), a virtual channel for observation of AuNPs by light diffraction.

Bai et al. [35] describe the formation of a crown on the surface of the nanoparticles due to the interaction with the proteins present in their physical environment. This formation directly intervenes in the distribution and internalization pathway of the nanoparticles; the corona-NP complex contains opsins on the surface which would facilitate its recognition by immune cells. Due to its size (30 nm), the entry mechanism would be phagocytosis [19]. However, the presence of proteins in the serum of the culture medium would create the formation of the crown directing its entry by receptor-mediated endocytosis.

According to Dey et al. [36] and Tomić et al.[37], the presence of AuNPs is found within endosomes/lysosomes after their internalization. In figure 15 the nanoparticles are grouped and outside the nuclei. Therefore, they would have the same location. Although nanoparticles are inert and biocompatible, they can generate levels of cytotoxicity related to small sizes due to their higher surface ratio in intra or extracellular interactions. For example: Nps of 10 nm induce apoptosis in weak dendritic cells, while that Nps of 50 nm do not have cytotoxic effects even when using high concentrations of up to 200 ug / mL. Local or systemic toxicity can affect the interaction between the cell and the PN, however, the levels generated are lower compared to other types of nanoparticles that cause damage to genetic material [19].

IV. Conclusion

According to the expression of the p53 gene and cell viability analysis, 5 ug/mL of AuNPs is toxic to cells. However, $1.39x10^6$ mature dendritic cells were recorded for this concentration since they did not proliferate and had only differentiated. The differentiated and mature dendritic cells with AuNPs belong to the group of cCD1. This was determined by the characterization of co-stimulation factors CD40, CD86, and CD11c, in addition to the presence of MHC-II and CIITA, the maturation and activation of the DCs in contact with AuNPs, in 12.5 and 25 ug / mL concentration.

Scanning electron microscope confirmed its morphological state. Micrographs obtained from confocal microscopy revealed the presence of nanoparticles within cells, close to the nuclei, possibly within endosomes. In dendritic cells, gold nanoparticles have been involved in immunomodulation, helping to improve antigen uptake and intracellular targeting, or they can be functionalized as molecules of interest and generate effective targeting to enhance absorption and initiation of adaptive immunity [19]. Therefore, *in vivo* tests should be carried out with the dendritic cells obtained for the implementation of a possible immunomodulatory therapy. In addition, the effects related to the route of administration, distribution, metabolism, and excretion of gold nanoparticles should be investigated.

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