Hindawi Publishing Corporation
Journal of Nanomaterials
Volume 2016, Article ID 4176212, 8 pages
http://dx.doi.org/10.1155/2016/4176212

Research Article

In Vitro Evaluation of Colloidal Silver on Immune Function: Antilymphoproliferative Activity

M. A. Franco-Molina, E. Mendoza-Gamboa, D. G. Zarate-Triviño, E. E. Coronado-Cerda, J. M. Alcocer-González, D. Resendez-Pérez, M. C. Rodríguez-Salazar, L. G. Rivera-Morales, R. Tamez-Guerra, and C. Rodríguez-Padilla

Laboratory of Immunology and Virology, Faculty of Biological Sciences, Autonomous University of Nuevo Leon (UANL), P.O. Box 46, 66455 San Nicolás de los Garza, NL, Mexico

Correspondence should be addressed to M. A. Franco-Molina; moyfranco@gmail.com

Received 22 August 2016; Revised 15 November 2016; Accepted 6 December 2016

Academic Editor: Jorge Pérez-Juste

Colloidal silver (AgC) is currently used by humans and it can be internalized through inhalation, injection, ingestion, and dermal contact. However, there is limited information about immunological activity; more investigations using colloidal silver are needed. In the present study, the effects of AgC (17.5 ng/mL) on immunological parameters (proliferation and immunophenotyping) using human peripheral blood mononuclear cells (PBMC) and macrophages (phagocytosis) and cytotoxicity on leukemia and lymphoma cancer cell lines (1.75 to 17.5 ng/mL) were investigated. AgC was observed to significantly ($p < 0.05$) decrease interleukin-2 (IL-2) production and proliferation induced by phytohemagglutinin or concanavalin A in PBMC without affecting its cell viability but with cytotoxic effect on cancer cells. IL-2, IL-4, IL-6, IL-10, INF-γ, and IL-17A cytokines production and $CD3^+$, $CD3^-CD19^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, and $CD16^+CD56^+$ PBMC phenotypes were not affected by AgC. The present study demonstrates that colloidal silver is harmless and nontoxic to the immune system cells and its ability to interfere with the immune response by decreasing cell proliferation when stimulated with mitogens demonstrated the antilymphoproliferative potential of AgC.

1. Introduction

Bioactivity of substances has been screened to identify properties related to the antitumoral or antiproliferative action [1]. Products with this activity have been used in clinical practice for the treatment of cancer or diseases related to inflammation such as autoimmunity. Silver products have been used for thousands of years for hygiene and as antimicrobial agents. Since 1964, colloidal silver (AgC) has been registered as a biocidal material in the United States [2]; in Mexico, AgC is commonly used as disinfectant of water and food for human consumption [3]. Generally, these products are a mix of metallic nanoparticles with oxidation state of zero (Ag^{+0}) and silver salts with oxidation states of Ag^{+1}, +2, or +3 [4]. There are studies indicating that the antibacterial [5] and antitumor properties depend on the oxidation states of silver [3]. Silver nanoparticles (AgNPs) and silver ions (Ag^+) have different levels of toxicity due to their superficial charge interactions. Silver ions are more toxic because they can interact with negative groups of proteins, producing structural changes on the cellular membrane and cytoplasmic proteins, while AgNPs can interact with DNA, causing damage and structural blocking; some studies have proposed that these nanostructures can produce increased toxicity at long exposition times due to the fact that AgNPs in aqueous solutions can oxidize and release silver ions; the successful use of colloidal silver solutions can be explained by this action mechanism [6, 7]. The anticancer activity of AgC on MCF-7 cancer cells is probably due to induced apoptosis by decreasing lactate dehydrogenase and increasing superoxide dismutase activities; in contrast, in PBMC, lactate dehydrogenase activity decreased with AgC, but it did not correlate with cell death [3]. There is scarce scientific information about immunological and cancer parameters in humans regarding the effects of colloidal silver, as a mix of nanoparticles and ions, compared to silver nanoparticles

research. The present study was designed to explore the antiproliferative properties of colloidal silver and its effect on cellular immunophenotyping, cytokines production, and phagocytosis on PBMC.

2. Materials and Methods

2.1. Colloidal Silver (AgC). Grenetine-stabilized colloidal silver was purchased from MICRODYN (México) as a 0.35% stock solution. It was sterilized by filtration (0.2 μm filter, Millipore, USA) and diluted to a concentration of 10.5 μg/mL with RPMI-1640, supplemented with 10% FBS for in vitro assays.

2.2. Reagents. Phytohemagglutinin (used at doses of 5 μg/mL) and concanavalin A (used at doses of 5 μg/mL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Doxorubicin (LEMERY S.A. de C.V., México) was stored as 3.4 mM stock solution in RPMI 1640 at room temperature and LPS (*E. coli* 0111:B4, Sigma-Aldrich) was prepared at 10 ng/mL to treat human peripheral blood mononuclear cells.

2.3. AgC Characterization. The morphology of colloidal silver was investigated with field emission scanning electron microscope (SEM) (Nova NanoSEM200 FEI). Colloidal solution (50 μL) was placed on a carbon tape and dried at room temperature. Nanoparticle size and distribution measurements were determined using a dynamic light scattering (DLS) performed by a nanosizer NS 90 Malvern Instruments (Malvern Instruments, UK); size distributions given by DLS were reported as percentage of intensity, and results were presented as the mean value of at least three measurements. Resonance plasmon measured by UV-vis was observed in a range between 300 and 600 nm using NanoDrop Spectrophotometer 2000 (Thermo Fisher Scientific).

2.4. Isolation of Peripheral Blood Mononuclear Cells (PBMC). Blood from healthy human volunteers was obtained with heparinized syringes and placed into sterile polypropylene tubes. PBMC were further isolated with Histopaque 1.077 density gradient centrifugation at 400 g for 30 min at 25°C (Sigma-Aldrich). PBMC were washed twice with RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution (referred as complete RPMI medium) at 250 g for 10 min at 25°C.

2.5. Cell Viability. PBMC were adjusted to 1×10^6 cells/mL in complete RPMI medium, and 17.5 ng/mL of colloidal silver was added and incubated for 72 h at 37°C and 5% CO_2 atmosphere. Thereafter, the supernatant was removed by centrifugation at 250 g. Cells were then washed twice with complete RPMI medium. Cell viability was determined by the colorimetric MTT reduction assay, and optical densities resulting from formazan production were read at 570 nm. Cell viability was expressed as percentage viability compared with the untreated control. Results were given as the mean ± SD of three independent experiments.

K562 (chronic myelogenous leukemia), MOLT-4 (acute lymphoblastic leukemia), Ramos (Burkitt's lymphoma), and L5178Y (lymphoma) cancer cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and maintained in complete RPMI medium. Cells were grown at 37°C and 5% CO_2 atmosphere. Cancer cell lines (5×10^3 cells/well) were plated on 96-well plates and incubated for 24 h at 37°C in 5% CO_2 atmosphere.

After incubation, the culture medium was removed, and colloidal silver was added at concentrations ranging from 1.75 to 17.5 ng/mL. The plates were then incubated for 5 h at 37°C and 5% CO_2 atmosphere. Thereafter, the supernatant was removed and cells were washed twice with RPMI 1640 medium. Cell viability was determined by the trypan blue exclusion method, and cytotoxicity was expressed as 50% (LD_{50}) and 100% (LD_{100}) cell growth inhibition, compared with untreated control. Results were given as the mean ± SD of three independent experiments.

2.6. Cytokines Assay. Supernatants from AgC-treated PBMC were analyzed for cytokines levels. IL-2, IL-4, IL-6, IL-10, IFN-γ, TNF-α, and IL-17A human cytokines were determined by flow cytometry (Accuri C6, BD Bioscience, CA, USA), using CBA Th1/Th2/Th17 Cytokine Kit BD™ (BD Bioscience, Bedford, MA, USA), following manufacturer's instructions. CBA Flex Set analysis was performed using FCAP array v1.0 software (Soft Flow Inc., USA). Protein values were converted to NIBSC/WHO protein standards for further comparisons.

2.7. Proliferation and IL-2 Assay. PBMC were isolated by Histopaque density gradient centrifugation, washed three times with PBS, and resuspended in Diluent C at 10^6 cells/mL. The cell suspension was then diluted with the same volume of 2.5 mM PKH26 dye stock (Sigma, St. Louis, MO) prepared in Diluent C, incubated for 3 min at room temperature, then added to an equal volume of FBS, and incubated at room temperature for a further 2 min to stop the labeling, according to "Rimaniol et al., 2003 [8]." Thereafter, PBMC were adjusted at concentrations of 1×10^6 cells/mL, treated with colloidal silver at a concentration of 17.5 ng/mL, PHA or Con A, and incubated for 72 h at 37°C and 5% CO_2 atmosphere; the cellular proliferation was determined by flow cytometry. The amounts of IL-2 content in supernatants of PBMC were measured by Human IL-2 High Sensitivity ELISA kit (limit of detection 0.4 pg/mL) and used according instructions of manufacturer (eBiosciences, Vienna, Austria).

2.8. Determination of Nitrite/Nitrate Production. Nitrites and nitrates levels were measured by the colorimetric Griess reaction using nitrate reductase (Nitrate/Nitrite Colorimetric Assay Kit Cayman, USA) according to the manufacturer's instructions. Serum nitrite/nitrate levels were expressed as nM/200 μL.

2.9. Flow Cytometry Analysis of Cell Surface Antigens. PBMC were adjusted at concentrations of 1×10^6 cells/mL and treated with colloidal silver at the concentration of 17.5 ng/mL, and

the plates were incubated for 72 h at 37°C and 5% CO_2 atmosphere. Thereafter, cells were collected by centrifugation at 600 g for 10 minutes and one set of antibodies $CD3^+$ FITC/$CD8^+$, PE/$CD45^+$, and PerCP/CD4 APC or another set of antibodies $CD3^+$ FITC/$CD16^+$, CD56 PE/CD45, and PerCP/CD19 APC (BD Multitest IMK Kit) were added and incubated 15 minutes at room temperature in the dark. The erythrocytes were then lysed by adding 450 μL of 1x BD FACS™ lysing solution and incubating 15 minutes at room temperature in the dark. Samples were then ready to be analyzed on the cytometer. The acquisition was performed on Accuri C6 BD instrument using BD Multiset software with BD Worklist Manager software. Automated gating was used for easy analysis of each subset population.

2.10. FITC-Dextran Uptake. Dendritic cells (DCs) were generated from PBMC and allowed to adhere to 0.22 μm filter-capped culture flasks (TPP, Germany). After 2 h at 37°C nonadherent cells were removed, and adherent monocytes were subsequently cultured for 5 days in RPMI-1640 supplemented with 10% FBS and 800 U/mL rhuGM-CSF (Peprotech, México) and 50 ng/mL IL-4 (Peprotech, México). Thereafter, cells were treated with colloidal silver at concentrations of 17.5 ng/mL and incubated for 72 h at 37°C and 5% CO_2 atmosphere. DCs endocytosis was evaluated by incubating 1×10^6 cells with 1 mg/mL FITC-Dextran (BD) for 30 min at 37°C. After washing, cells were analyzed by flow cytometry. Controls included tubes incubated with FITC-Dextran at 4°C to inhibit the endocytic process and a basal uptake performed at the 0-time point. Uptake was quantified by FACS analysis (10,000 cells per point) [5]. Viability was determined by the trypan blue exclusion technique.

2.11. Statistical Analysis. The results were evaluated by ANOVA and median cytokines levels between treatments were compared using the Mann–Whitney U test.

3. Results

3.1. Colloidal Silver Characterization. The characterization of colloidal silver dissolved in water and cell culture medium was analyzed by dynamic light scattering (DLS); colloidal silver dissolved in water showed an average size of 100 nm with a polydispersity index of 0.2; colloidal silver dissolved in cell culture medium showed an average size of 155 nm and polydispersity index of 0.23 (Figures 1(a) and 1(b)). SEM image showed particles population dissolved in water with particle size between 50 and 190 nm (Figures 1(c) and 1(d)); colloidal silver dissolved in cell culture medium showed a combination of nanoparticles and some silver salts (Figures 1(e) and 1(f)); however, the average size obtained by DLS correlated with histogram of particles and characteristic plasmon resonance (Figures 1(g), 1(h), and 1(i)). The analysis of colloidal silver suggests that this solution has semispherical shape and heterogeneous population, formed by nanoparticles and cluster formed by silver salts. Once the AgC was characterized, we proceeded to evaluate the different biological effects.

3.2. Determination of Cellular Proliferation and IL-2 Production. First, we determined whether the cytotoxic dose previously used in breast cancer cells [3] affects lymphocytes or macrophages functions. The results showed that colloidal silver treatment did not affect significantly ($p = 0.231$) PBMC cell proliferation and cell count, and the treatments with mitogens Con A or PHA significantly ($p = 0.05$) increased the cell proliferation and cell count, compared with the untreated control; interestingly, the combined treatments with AgC/Con A or AgC/PHA significantly ($p = 0.05$) decreased the cell proliferation and cell count (Figures 2 and 3) of PBMC. Similar results were observed by flow cytometry and fluorescence microscopy using the fluorescent dye PKH26 (control (94%), Con A (165%), PHA (156%), AgC (91%), AgC + Con A (80%), and AgC + PHA (77%)) (Figures 3, 4, and 5). In addition, AgC treatment did not affect the IL-2 production when compared with the control (15 pg/mL) ($p = 0.85$), but an increase in IL-2 production was found when PBMC were stimulated with PHA (176 pg/mL) or Con A (150 pg/mL), and treatments with AgC + Con A (15 pg/mL) or AgC + PHA (13 pg/mL) decreased IL-2 production ($p = 0.05$) (Figure 4).

3.3. Cytotoxic Effect of AgC on Lymphoid and Leukemia Cancer Cell Lines. Our results confirmed the antiproliferative and cytotoxic properties of AgC on leukemic (Molt-4 and K562) and lymphoma cell lines (Ramos and L5178Y) in a dose-dependent manner (1.75 to 17.5 ng/mL) ($p = 0.03$) (Figure 6).

3.4. Cytokine Determination. Besides AgC treatment did not affect the production ($p < 0.001$) of IL-2, IL-4, IL-6, IL-10, IFN-γ, IL-17A (0 pg/mL), or TNF-α (5.84 pg/mL), compared with untreated control. However, LPS treatment used as positive control induced a high production of all cytokines evaluated (Table 1).

3.5. Phenotyping of Cell Surface Markers. Our results demonstrated that AgC treatment did not affect the percentage of expression of cellular populations $CD3^+$ (79.7%), $CD3^-CD19^+$ (10.2%), $CD3^+CD4^+$ (52.2%), $CD3^+CD8^+$ (33.9%), and $CD16^+CD56^+$ (7.6%), compared with the control ($p < 0.001$) (Table 2).

3.6. Peroxynitrites and Uptake of FITC-Dextran Determinations. The colloidal silver treatment (99%) did not affect the viability of dendritic cells (Figure 7(a)) or the levels of peroxynitrites evaluated (Figure 7(b)) but increased the phagocytosis percentage (Figure 7(c)), compared with the control ($p < 0.001$).

4. Discussion

It is known that several chemotherapeutic agents used in cancer treatment (cyclophosphamide, vinblastine, vincristine, bleomycin, and cisplatinum) have antiproliferative and cytotoxic effects; but at low doses they can stimulate the immune response [9]. The effect on the immune system of any substance should be tested because any damage

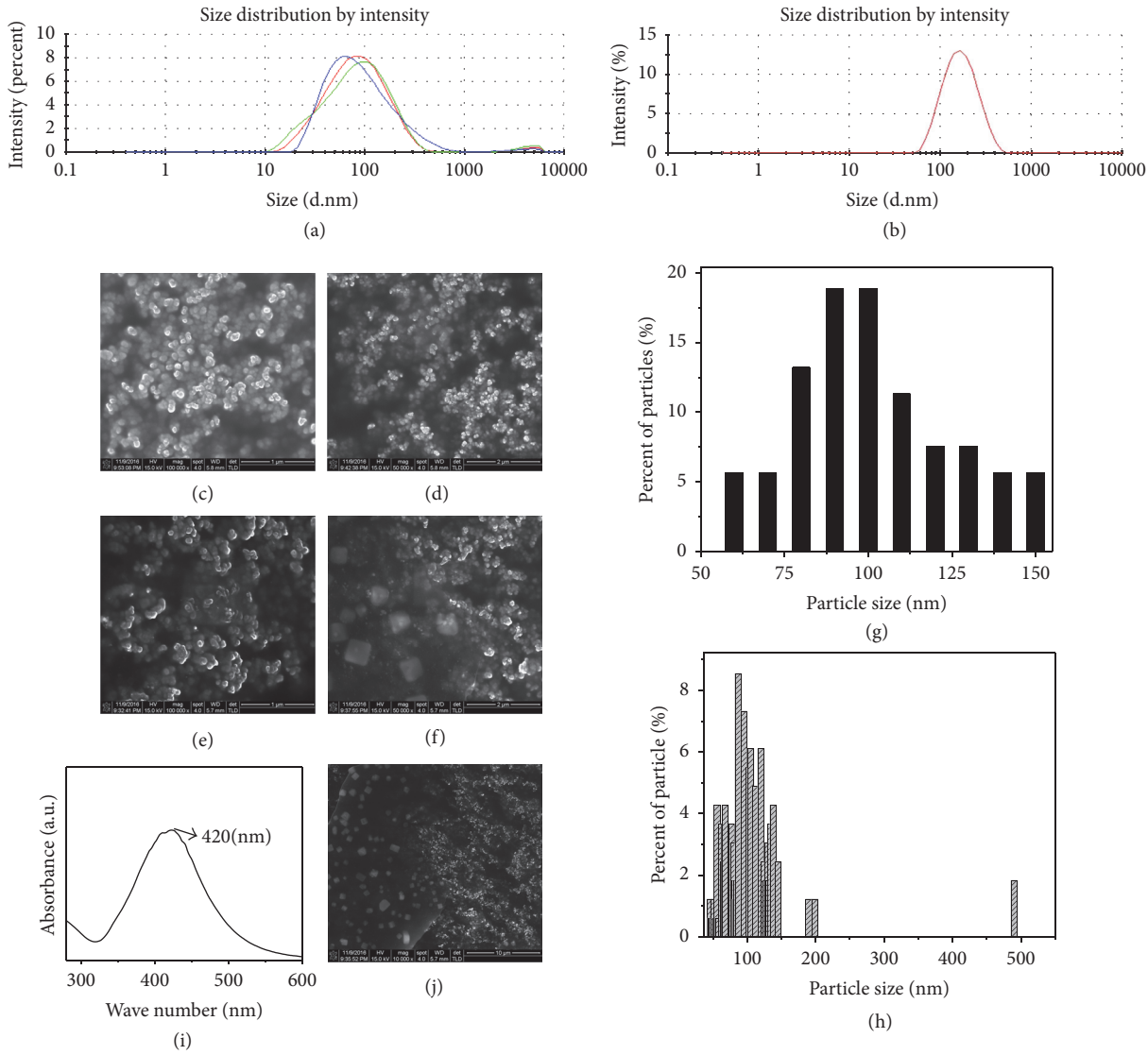

Figure 1: Size distribution of colloidal silver. (a) DLS of colloidal silver dissolved in water showed an average size of 100 nm with a polydispersity index of 0.2. (b) DLS measurements of colloidal silver dissolved in cell culture medium with an average size of 155 nm and polydispersity index of 0.23. (c, d) SEM image of particles populations dissolved in water. (e, f) SEM image of colloidal silver dissolved in cell culture medium. (g, h) Histogram of particles dissolved in water and culture medium, respectively. (i) UV-vis spectra of colloidal silver. (j) Silver salts formed in samples of colloidal silver dissolved in culture medium. DLS, dynamic light scattering; SEM, scanning electron microscopy; and a.u., arbitrary units.

Table 1: Cytokines determination in human PBMC, treated with AgC.

	PBMC cytokines production (pg/mL)						
Treatments	IL-2	IL-4	IL-6	IL-10	TNF	INF-γ	IL-17A
Control	0	0	0	0	7.07	0	0
AgC	0	0	0	0	5.84	0	0
LPS	11.69**	109.31**	15.14**	4.18**	1222.03**	0	3.17**

Notes. Total production of IL-2, IL-4, IL-6, IL-10, TNF, INF-γ, and IL-17A, after treatment with AgC or LPS, used as positive control. The data represent the mean ± standard deviation of three independent experiments. ** $p < 0.05$. AgC, colloidal silver; LPS, lipopolysaccharide.

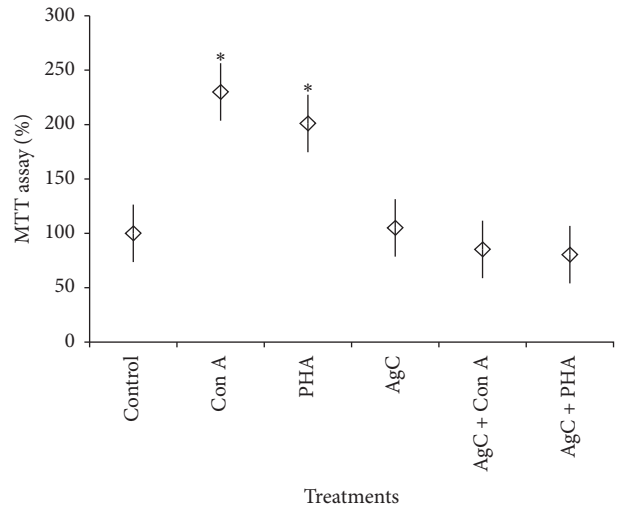

Figure 2: Cell viability of PBMC treated with colloidal silver and mitogens. PBMC (1×10^6 cells/mL) were treated with Con A (5 μg/mL), PHA (5 μg/mL), AgC (17.5 ng/mL), AgC (17.5 ng/mL) + Con A (5 μg/mL), or AgC (17.5 ng/mL) + PHA (5 μg/mL) and incubated for 72 h at 37°C and 5% CO_2 atmosphere. Cell viability was analyzed by MTT assay. The data represent the mean ± standard deviation of three independent experiments. $^*p < 0.05$. AgC, colloidal silver; PHA, phytohemagglutinin; and Con A, concanavalin A.

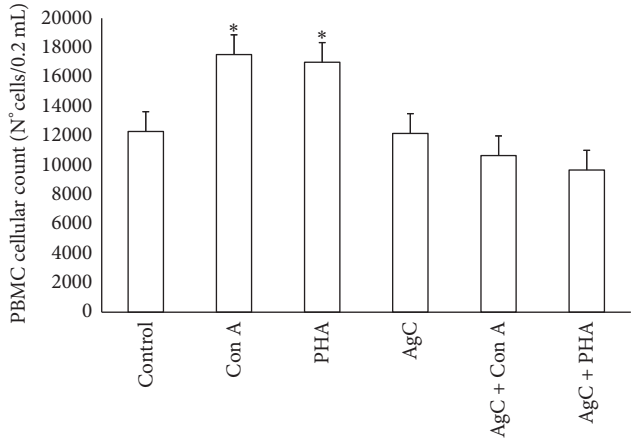

Figure 3: Cellular count of PBMC treated with colloidal silver and mitogens. PBMC (1×10^6 cells/mL) were treated with Con A (5 μg/mL), PHA (5 μg/mL), AgC (17.5 ng/mL), AgC (17.5 ng/mL) + Con A (5 μg/mL), or AgC (17.5 ng/mL) + PHA (5 μg/mL) and incubated for 72 h at 37°C and 5% CO_2 atmosphere. Cell count was analyzed by flow cytometry. The data represent the mean ± standard deviation of three independent experiments. $^*p < 0.05$. AgC, colloidal silver; PHA, phytohemagglutinin; and Con A, concanavalin A.

Table 2: Phenotypic characterization of lymphoid human PBMC subsets.

Phenotype	Control (%)	AgC (%)
$CD3^+$	84.8	79.7
$CD3^-CD19^+$	7.0	10.2
$CD3^+CD4^+$	51.8	52.2
$CD3^+CD8^+$	32.7	33.9
$CD16^+CD56^+$	6.4	7.6

Notes. Representative flow cytometric analysis of lymphoid subsets from PBMC. The data represent the mean ± standard deviation of three independent experiments. AgC, colloidal silver.

in this system could affect the body homeostasis. In the present study the analysis of colloidal silver suggests the presence of a heterogeneous population of nanoparticles and clusters formed by silver salts, probably originated from interactions with proteins contained in complete culture medium. The aggregation effect of the silver particles in biological fluids due to the presence of proteins and lipids has been reported [7]. In addition our results showed that AgC (17.5 ng/mL) can inhibit the production of IL-2 induced by mitogens. Hence, the immunosuppression induced in PBMC could be mediated by the inhibition of IL-2 release. The immunosuppressant activity of some drugs such as cyclosporine and azathioprine has been corroborated by the inhibition of proliferation of lymphocytes and production of cytokines (INF-γ, IL-2, RANTES, TGF-β, and TNF-α), when lymphocytes are stimulated by reagents such as PHA, Con A, or pokeweed mitogen [10]. IL-2 is an autocrine

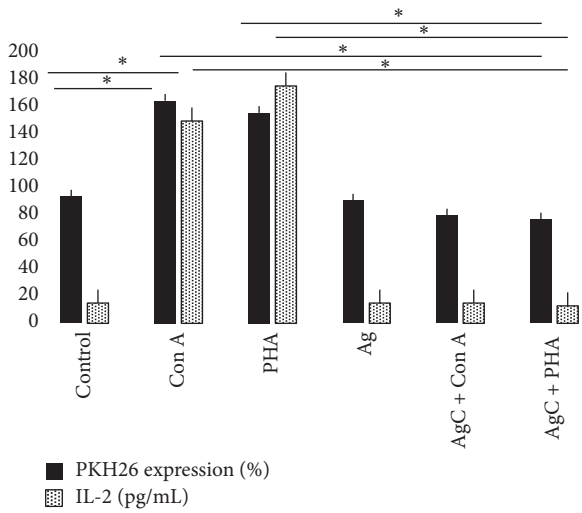

Figure 4: Cellular proliferation and IL-2 production of PBMC treated with colloidal silver and mitogens. PBMC (1×10^6 cells/mL) were treated with Con A (5 μg/mL), PHA (5 μg/mL), AgC (17.5 ng/mL), AgC (17.5 ng/mL) + PHA (5 μg/mL), or AgC (17.5 ng/mL) + Con A (5 μg/mL) and incubated for 72 h at 37°C and 5% CO_2 atmosphere. Cellular proliferation based on the expression of PKH26 was analyzed by flow cytometry. The IL-2 supernatants were evaluated by ELISA test. The data represent the mean ± standard deviation of three independent experiments. $^*p < 0.05$. AgC, colloidal silver; PHA, phytohemagglutinin; and Con A, concanavalin A.

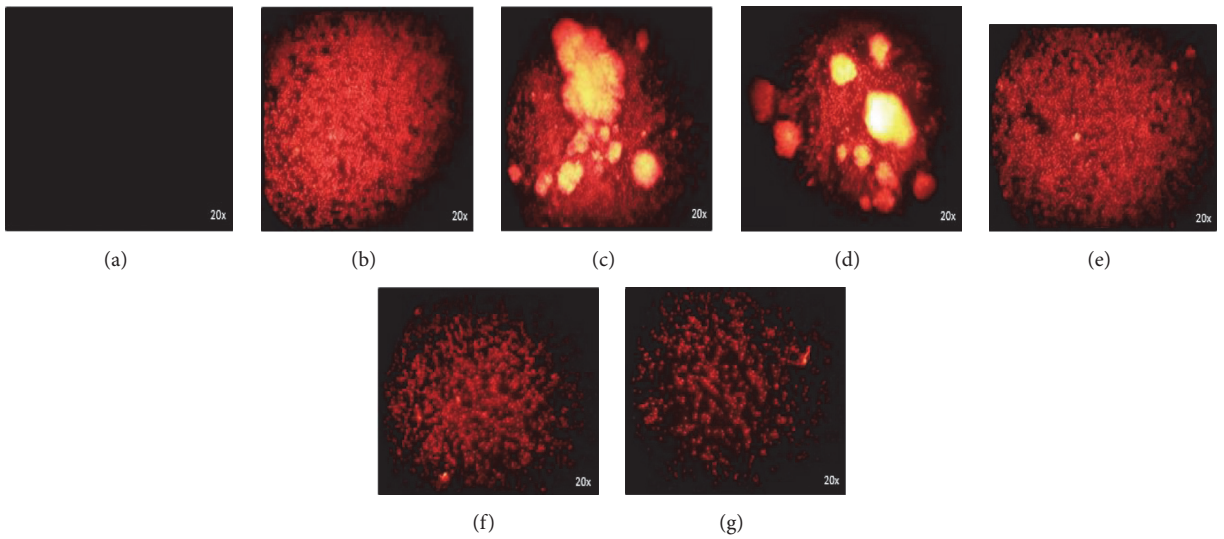

Figure 5: Cellular proliferation of PBMC treated with colloidal silver and mitogens. PBMC (1×10^6 cells/mL) were treated with (a) negative control, (b) control, (c) PHA (5 μg/mL), (d) Con A (5 μg/mL), (e) AgC (17.5 ng/mL), (f) AgC (17.5 ng/mL) + PHA (5 μg/mL), or (g) AgC (17.5 ng/mL) + Con A (5 μg/mL) and incubated for 72 h at 37°C and 5% CO_2 atmosphere. Cellular proliferation was analyzed by microscopy fluorescence. AgC, colloidal silver; PHA, phytohemagglutinin; and Con A, concanavalin A.

growth factor for T cells [11] and its production has been selectively inhibited by the treatment with the immunosuppressants tacrolimus (FK506) or mycophenolic acid [10]. It is known that immunosuppressant agents (corticosteroids) are used for the treatment of lymphoid malignancies because they induce apoptosis of malignant lymphoid cells [12]. We demonstrated the antiproliferative and cytotoxic properties of AgC (155 nm) (doses ranging from 1.75 to 17.5 ng/mL) on leukemic and lymphomatous cell lines despite previous reports which mentioned that silver particles in the range of 10–100 nm of diameters are more cytotoxic than bigger size particles [13]. It is necessary to know the mechanism of selectivity between PBMC and myeloid and lymphoid origin cancer cells and future studies should be developed in the area of receptor-mediated apoptosis such as discussed by "Vega and De Maio, 2005 [14]." Because of glucocorticoid resistance in lymphoma treatment, the tumor cells continue their expansions in the presence of glucocorticoids [12]. For this reason AgC could offer a new clinical option to be considered but more studies related are needed. On the other

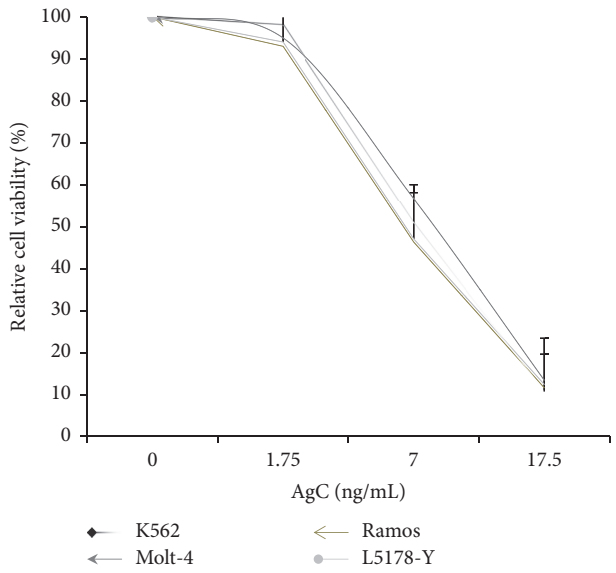

Figure 6: Cell viability of cancer cell lines treated with colloidal silver and mitogens. K562, Molt-4, Ramos, and L5178Y cancer cell lines (5×10^3 cells/well) were treated with several doses of AgC and incubated for 5 h at 37°C and 5% CO_2 atmosphere. Cell viability was analyzed by MTT assay. The data represent the mean ± standard deviation of three independent experiments. $^*p < 0.05$. AgC, colloidal silver.

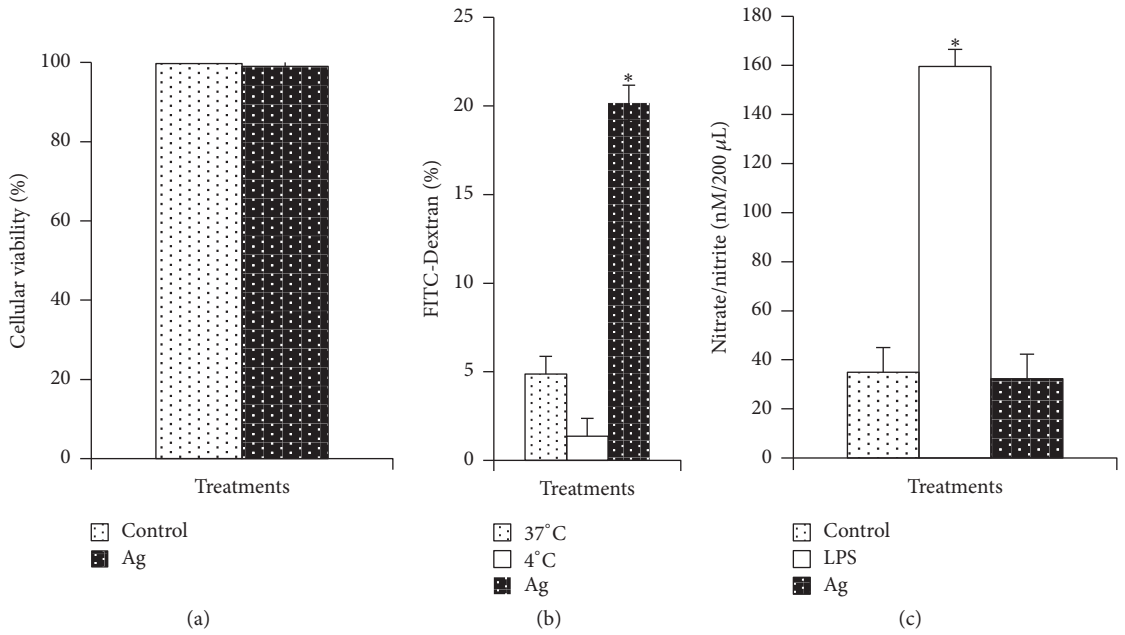

Figure 7: Cell viability of human macrophages, phagocytosis, and peroxynitrites production. Human macrophages (1×10^6 cells) were treated with AgC and incubated for 5 h at 37°C and 5% CO_2 atmosphere. (a) Cell viability was analyzed by trypan blue assay. (b) Macrophages phagocytosis of FITC-Dextran evaluated by flow cytometry. (c) Nitrate/nitrite determined by Griess reaction (nitrate/nitrite colorimetric assay kit); the LPS was used as positive control. The data represent the mean ± standard deviation of three independent experiments. $^*p < 0.05$. AgC, colloidal silver; FITC-Dextran, fluorescein isothiocyanate-Dextran; and LPS, lipopolysaccharide.

hand, our results indicate that cytokines production and the percentage of surface markers expressed by T, B, and NK cells are not affected in response to AgC (17.5 ng/mL), opposite to other immunosuppressors such as rapamycin or soraphen, for which the immunosuppressor effect is attributed to the interference of a signaling pathway and the metabolism of fatty acids [15]. Furthermore, there is evidence that immune system cells can be activated in response to biomaterials although MHC/peptide/TCR induced signaling pathways are not expected. However, alternative noncognate pathways can lead to activation by cross-linking glycoproteins on the plasma membrane surface such as mitogen induced

lymphocyte proliferation [16]. With regard to peroxynitrites, colloidal silver (17.5 ng/mL) did not induce their production, but phagocytosis activity increased probably due to the uptake of colloidal silver in an unspecific manner. Other authors have described that silver nanoparticles in a range of 5, 10, 15, and 100 nm increase the reactive oxygen species affecting the mitochondrial function [17] and that phagocytosis of silver particles blocks cell cycle in the S-phase and stimulates inflammatory signaling through generation of reactive oxygen species, followed by secretion of TNF-α, which decreased the viability of rat liver cells [18].

In conclusion, the present study demonstrates the nontoxicity of colloidal silver over immune system cells and its ability to interfere with the immune response by decreasing cell proliferation when stimulated with mitogens, suggesting that colloidal silver can be considered as an immunosuppressive agent, but more studies must be performed to establish its effectiveness and mechanism of action.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

This study is supported by Laboratory of Immunology and Virology, Faculty of Biological Sciences, Autonomous University of Nuevo Leon, San Nicolás de los Garza, NL, Mexico, in collaboration with "Red Temática de Inmunología en Cáncer y Enfermedades Infecciosas" with Register no. 253053, CONACYT.

References

[1] F.-Y. Chang and S. F. Brady, "Discovery of indolotryptoline antiproliferative agents by homology-guided metagenomic screening," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 7, pp. 2478–2483, 2013.

[2] Pyramed Colloidal Silver Generators, "Colloidal Silver History and Nanosilver Technology," SciTron Healing, https://colloidal-silvergenerator.co.uk/colloidal-silver-benefits/colloidal-silver-history/.

[3] M. A. Franco-Molina, E. Mendoza-Gamboa, C. A. Sierra-Rivera et al., "Antitumor activity of colloidal silver on MCF-7 human breast cancer cells," *Journal of Experimental & Clinical Cancer Research*, vol. 29, no. 1, article no. 148, 2010.

[4] B. Reidy, A. Haase, A. Luch, K. A. Dawson, and I. Lynch, "Mechanisms of silver nanoparticle release, transformation and toxicity: a critical review of current knowledge and recommendations for future studies and applications," *Materials*, vol. 6, no. 6, pp. 2295–2350, 2013.

[5] E. M. von Euw, M. M. Barrio, D. Furman et al., "Monocyte-derived dendritic cells loaded with a mixture of apoptotic/necrotic melanoma cells efficiently cross-present gp100 and MART-1 antigens to specific $CD8^+$ T lymphocytes," *Journal of Translational Medicine*, vol. 5, article 19, 2007.

[6] Z.-M. Xiu, Q.-B. Zhang, H. L. Puppala, V. L. Colvin, and P. J. J. Alvarez, "Negligible particle-specific antibacterial activity of silver nanoparticles," *Nano Letters*, vol. 12, no. 8, pp. 4271–4275, 2012.

[7] R. Behra, L. Sigg, M. J. Clift et al., "Bioavailability of silver nanoparticles and ions: from a chemical and biochemical perspective," *Journal of The Royal Society Interface*, vol. 10, no. 87, 2013.

[8] A. C. Rimaniol, G. Garcia, S. J. Till et al., "Evaluation of CD4+ T cells proliferating to grass pollen in seasonal allergic subjects by flow cytometry," *Clinical and Experimental Immunology*, vol. 132, no. 1, pp. 76–80, 2003.

[9] E. Mihich, "On the immunomodulating effects of anti-cancer drugs and their therapeutic exploitation," *Japanese Journal of Clinical Oncology*, vol. 30, no. 11, pp. 469–471, 2000.

[10] W. Ai, H. Li, N. Song, L. Li, and H. Chen, "Optimal method to stimulate cytokine production and its use in immunotoxicity assessment," *International Journal of Environmental Research and Public Health*, vol. 10, no. 9, pp. 3834–3842, 2013.

[11] A. S. Kennell, K. G. Gould, and M. R. Salaman, "Proliferation assay amplification by IL-2 in model primary and recall antigen systems," *BMC Research Notes*, vol. 7, no. 1, article 662, 2014.

[12] M. Ohe, S. Hashino, and A. Hattori, "Successful treatment of diffuse large B-cell lymphoma with clarithromycin and prednisolone," *Korean Journal of Hematology*, vol. 47, no. 4, pp. 293–297, 2012.

[13] T. A. J. Souza, L. P. Franchi, L. R. Rosa, M. A. M. S. da Veiga, and C. S. Takahashi, "Cytotoxicity and genotoxicity of silver nanoparticles of different sizes in CHO-K1 and CHO-XRS5 cell lines," *Mutation Research—Genetic Toxicology and Environmental Mutagenesis*, vol. 795, pp. 70–83, 2016.

[14] V. L. Vega and A. De Maio, "Increase in phagocytosis after geldanamycin treatment or heat shock: role of heat shock proteins," *Journal of Immunology*, vol. 175, no. 8, pp. 5280–5287, 2005.

[15] C. N. Castro, J. Freitag, L. Berod, M. Lochner, and T. Sparwasser, "Microbe-associated immunomodulatory metabolites: influence on T cell fate and function," *Molecular Immunology*, vol. 68, no. 2, pp. 575–584, 2015.

[16] H. Miyazaki, A. Kikuchi, Y. Koyama, T. Okano, Y. Sakurai, and K. Kataoka, "Boronate-containing polymer as novel mitogen for lymphocytes," *Biochemical and Biophysical Research Communications*, vol. 195, no. 2, pp. 829–836, 1993.

[17] J. W. Han, S. Gurunathan, J.-K. Jeong et al., "Oxidative stress mediated cytotoxicity of biologically synthesized silver nanoparticles in human lung epithelial adenocarcinoma cell line," *Nanoscale Research Letters*, vol. 9, no. 1, 2014.

[18] S. M. Hussain, K. L. Hess, J. M. Gearhart, K. T. Geiss, and J. J. Schlager, "In vitro toxicity of nanoparticles in BRL 3A rat liver cells," *Toxicology in Vitro*, vol. 19, no. 7, pp. 975–983, 2005.