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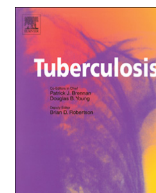

IMMUNOLOGICAL ASPECTS

Different responses of human mononuclear phagocyte populations to *Mycobacterium tuberculosis*

Camilo Duque [a], Leonar Arroyo [a], Héctor Ortega [b,c], Franco Montúfar [d], Blanca Ortíz [a], Mauricio Rojas [a], Luis F. Barrera [a,*]

[a] *Grupo de Inmunología Celular e Inmunogenética (GICIG), Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Antioquia, UdeA, Calle 70 No. 52-21, Medellín, Colombia*
[b] *Hospital Pablo Tobón Uribe, Medellín, Colombia*
[c] *Clínica Cardiovascular Santa María, Medellín, Colombia*
[d] *IPS Universitaria Clínica León XIII Sede Medellín, Medellín, Colombia*

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SUMMARY

Mycobacterium tuberculosis (*Mtb*) infects different populations of macrophages. Alveolar macrophages (AMs) are initially infected, and their response may contribute to controlling *Mtb* infection and dissemination. However, *Mtb* infection may disseminate to other tissues, infecting a wide variety of macrophages. Given the difficulty in obtaining AMs, monocyte-derived macrophages (MDMs) are used to model macrophage–mycobacteria interactions in humans. However, the response of other tissue macrophages to *Mtb* infection has been poorly explored. We have compared MDMs, AMs and splenic human macrophages (SMs) for their *in vitro* capacity to control *Mtb* growth, cytokine production, and induction of cell death in response to *Mtb* H37Rv, and the Colombian isolate UT205, and to the virulence factor ESAT-6. Significant differences in the magnitude of cell death and cytokine production depending mainly on the *Mtb* strain were observed; however, no major differences in the mycobacteriostatic/mycobacteriocidal activity were detected among the macrophage populations. Infection with the clinical isolate UT205 was associated with an increased cell death with membrane damage, particularly in IFNγ-treated SMs and H37Rv induced a higher production of cytokines compared to UT205. These results are concordant with the interpretation of a differential response to *Mtb* infection mainly depending upon the strain of *Mtb*.

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1. Introduction

Mycobacterium tuberculosis (*Mtb*) the etiological agent of tuberculosis (TB) uses both monocytes and macrophages as niches in order to prolong survival, multiplication and transmission in the human population. In opposition to this pathogen, mechanisms portrayed by macrophages have evolved in order to control or to eliminate the disturbing consequences of infection, such as chronic inflammation, pathology and disease.

Circulating monocytes give rise to specialized tissue macrophage populations, a process in which microenvironment has proposed to play a directive role [1]. In the current view, *Mtb* infects alveolar macrophages, which in turn trigger several signaling pathways resulting in a chemokine-dependent recruitment of circulating monocytes and T cells to the site of infection and the production of proinflammatory cytokines, including IFNγ, TNFα, IL-1β, IL-6, and IL-18 aimed to the elimination of the invading pathogen. Moreover, anti-inflammatory cytokines, such as IL-10 limits the inflammatory reaction [2]. However, in the majority of occasions, infection may persist for unlimited time, and in a minority of cases progress to an uncontrolled state. This state is characterized by dissemination through the lung tissue, or more rarely, to other tissues, generating a life threatening disease which is most common in infants and immunocompromised individuals [3,4].

Most of the knowledge we already have on the interaction of human macrophages and *Mtb* has been obtained from the use of monocyte-derived macrophages (MDMs). Blood derived monocytes are cultured *in vitro* for several days until they differentiate into cells resembling tissue macrophages. However, remarkably few studies have been performed with alveolar macrophages, the natural source of the primary infection, mostly due to ethical issues

* Corresponding author. Grupo de Inmunología Celular e Inmunogenética (GICIG), Instituto de Investigaciones Médicas, Facultad de Medicina, Universidad de Antioquia, Medellín, Colombia. Tel.: +57 4 219 6448; fax: +57 4 219 1060.
E-mail address: fbarrera@udea.edu.co (L.F. Barrera).

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related to the invasiveness of the procedure to obtain the samples of bronchoalveolar lavage. In addition, studies using other tissue macrophages are almost inexistent [5].

The genetic characterization and genome sequencing of *Mtb* strains has shown a greater variability than expected based on the clonal transmission of the bacilli. More interestingly, recent evidence suggests a phylogeographic adaptation of circulating *Mtb* strains to human populations that in some studies has been associated with different forms of TB disease [6]. Thus, more studies based on the interaction of monocytes and macrophage populations with circulating strains of *Mtb* are necessary to have a better understanding of the immune mechanisms associated with bacterial control.

In this study, we have compared the *in vitro* response of monocytes, MDMs, alveolar (AMs) and splenic macrophages (SMs) to infection with the laboratory strain of *Mtb* H37Rv and to the recently isolated Colombian strain of *Mtb* UT205. To examine the response of these mononuclear cells, the induction of cell death (apoptosis and necrosis) and production of cytokines in response to infection and to the virulence factor ESAT-6, and the antimycobacterial activity in the absence or presence of IFNγ were determined. Our results are consistent with a variable response of macrophages that mostly depends on the strain of *Mtb*.

2. Materials and Methods

2.1. Reagents

RPMI-1640, Heat inactivated AB Human Serum, Dulbecco's PBS were purchased from Invitrogen (Carlsbad, CA). Histopaque was purchased from Sigma (St. Louis, MO). Penicillin-streptomycin was purchased from Biowittaker (Walkersville, MD). Glycerol was purchased from Promega (Madison, WI).

2.2. Subjects

Healthy volunteers ($n = 13$) were used as a source of peripheral venous blood for obtaining monocytes and monocyte-derived macrophages (MDMs) as described below. Alveolar macrophages (AMs) were obtained from bronchoalveolar lavage (BAL) from individuals suspected of respiratory illnesses not associated with HIV-infection and/or malignancies of the myeloid system ($n = 8$) at the Hospital Universitario Pablo Tobón Uribe, Clínica Cardiovascular La María and IPS Universitaria Clínica León XIII, Sede Medellín (Medellín, Colombia). Splenic macrophages (SMs) were obtained from spleen slices from deceased donors ($n = 14$) of the Transplantation Programs of the Hospital Universitario Pablo Tobón Uribe, and the IPS Universitaria León XIII Sede Medellín (Medellín, Colombia). The cause of death for the majority of the donors ($n = 10$, 71.4%) was trauma; the remaining ($n = 4$, 28.6%), included different cerebrovascular causes. None of the donors were HIV^+.

2.3. Monocytes and macrophages

Monocytes were obtained from peripheral blood of healthy subjects and differentiated into MDMs as previously described [7]. To obtain monocyte monolayers, 2×10^5 $CD14^+$ cells were seeded in 0.5 ml of RPMI-1640 (Invitrogen, Grand Island, NY) supplemented with 0.5% AB^+ inactivated human serum (Invitrogen, Brown Deer, WI), penicillin and streptomycin (Biowittaker, Walkersville, MD), for 4 h at 37 °C, 5% CO_2, 95% relative humidity, and then extensively washed with DPBS (Invitrogen, Grand Island, NY) supplemented with 0.5% AB^+ inactivated human serum and antibiotics, prewarmed at 37 °C. In these conditions, $CD14^+$ cells represented >95% of the adherent cells (results not shown). MDMs were obtained after 5 days of culture of monocyte monolayers in RPMI-1640 supplemented with 10% AB^+ inactivated human serum, penicillin and streptomycin (complete medium, CM). Control experiments indicated no significant detachment of cells during the culture period (data not shown). Splenic macrophages were obtained of deceased donors as previously described [5]. Adherent cells were detached by treatment with 0.05% trypsin-EDTA for 10 min, washed, counted, and then seeded at 1×10^5 macrophages/well in 48-well tissue culture plates in CM without antibiotics for 24 h before infection. To prepare AMs, BAL obtained from healthy areas of the lung were centrifuged for 5 min at 650× *g* and resuspended in CM. One hundred thousand dark granular cells, morphologically corresponding to macrophages, were seeded in 48-well plates and cultured for 4 days in CM. At this point, nonadherent cells were eliminated by extensive washings with warm DPBS supplemented with 0.5% AB^+ human serum, and then cultured for additional 24 h in CM without antibiotics before being infected.

2.4. Mycobacteria

M. tuberculosis strain H37Rv was obtained from the Instituto Nacional de Salud, Bogotá, Colombia. The *Mtb* clinical isolates UT205, UT127 and UT379 were obtained from the Centro Colombiano para la Investigación en Tuberculosis (CCITB). *Mtb* was grown in Middlebrook 7H9 broth supplemented with 10% OADC (BD, NY) and Tween 80 (0.05%), for 2–3 weeks to reach exponential growth phase. Mycobacteria were cultured as previously described [8]. Mycobacterial clumps were disrupted by 6 sonication cycles of 10 s at 4 °C, each cycle for 40 W output (CV33 Sonics Vibra Cell, Newtown, CT). The sonicate was gently centrifuged for 5 min at 250× *g* at 4 °C, and the upper bacterial suspension was diluted in freezing medium, adjusted to final absorbance of 0.1 (OD_{620}) and frozen at −70 °C until used. The number of colony forming units (CFU) was determined by plating 20 μl of serial dilutions onto petri dishes (Corning, NY), containing Middlebrook 7H10 agar supplemented with glycerol and 10% OADC pH 7.2 and the CFU counted after 3 weeks of culture at 37 °C. Upon thawing, mycobacterial viability of FDA stained bacteria (usually more than 90%) was tested by flow cytometry essentially as described [9]. The Colombian strains of Mtb UT205, UT127 and UT379 were collected during a large cohort study conducted by the Centro Colombiano para la Investigación en Tuberculosis (CCITB) during 2005–2009 [10]. All of them belong to the Latinoamerican-Mediterranean family (LAM) of *Mtb* UT205 was obtained from a household in which an incident case was reported while no incident cases were reported in the household from which the UT127 strain was recovered although the household contacts showed evidence of infection as tested by an in-house IGRA. UT379 was recovered from a household in which no evidence of infection was detected in the household contacts.

2.5. Infection of macrophage populations

Macrophage populations (MDMs, SMs and AMs) were infected with *Mtb* at a multiplicity of infection (MOI) of 5:1 for 6 h, and washed extensively with warm DPBS supplemented with 0.5% AB^+ inactivated human serum to eliminate noningested bacteria. Twenty four hours after infection, macrophages were cultured in the presence or absence of recombinant human IFNγ (500 ng/ml). The effect of infection and IFNγ treatment on cell death and the mycobacteriostatic/mycobacteridal activity was determined

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