In vitro EFFECT OF **Mycobacterium Ieprae** SUSPENSIONS ON THE POLYMORPHONUCLEAR NEUTROPHILES FUNCTION OF HANSENÍASIS PATIENTS TO **Candida albicans** AND **Candida pseudotropicalis**

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ABSTRACT — The in vitro effect of **Mycobacterium Ieprae** suspensions on the PMN hability to phagocyting and killing **Candida albicans** and **Candida pseudotropicalls** was studied in forty-five patients of Hansen's disease and in fifteen healthy controls. Our results show no significative differences between the different studied groups, both for the phagocytosis and for the lysis of yeasts. There was no significant changes in the mean values of these functions after previous or simultaneously incubation with **Mycobacterium Ieprae** suspensions. Those observations confirmed that there are not alterations in the enzimatic battery of PMN in Hansen's disease patients and that the **Mycobacterium Ieprae** presence does not exert stimulating effect on this **in vitro** model.

Key *words*: *Mycobacterium leprae.* Neutrophiles. Hanseniasis. *Candida albicans. Candida pseudotropicalis.*

1. INTRODUCTION

The functions of the phagocytic system, postulated by Metchnicoff[19] at the end of the last century have been extensively studied, both in the subject concerning the mononuclear phagocytes and the polymorphonuclear neutrophiles (PMN), being their chemotactics mechanisms[4], those of ingestion of particles[16] and the enzimatic battery which takes part in their metabolism examined thoroughly[17].

As regard the polynuclear cells, it is assumed that their most important bactericidal mechanisms is the Klebanoff haloide-H202-myeloperoxidase system[15] which also reacts on viral particles and fungi. Another lytic system not connected with the above mentioned one has been specially studied mainly in monocytes, receiving the denomination of independent-myeloperoxidase or cationic protein system[17] being in agreement that it plays an important role in the phagocytic and lytic functions in the PMN.

In Hanseniasis (HD) patients, the phagocytic system was carefully examined because of being the *Mycobacterium leprae* an intracellular pathogen and having been proved the macrophagic condition of the Virchow's cells since early times. Most of the studies performed in HD patients were aimed at the peripheral blood monocytes, obtaining contradictory results, as it was reported by the different hives-

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tigators about the functional capacity in *vitro* of these cells facing M *leprae* and other microorganisms[1,2,3,5,7].On studying PMN, Goihman-Yahr *et ally*[12] reports the spontaneous reduction of Nitroblue-Tetrazolium (NBT) in cells of reactional lepromatous patients, as well as the existence of an increasing of this function of PMN in presence of *Mleprae* [13],

As far as Miranda is concerned, he refers the phagocytic activity of PMN facing *M leprae*[20]; *in* a previous paper, one of us studied the capacity of PMN in HD patients in order to phagocyte and destroy two kinds of yeasts: *Candida albicans* and *Candida pseudotropicalis,* not finding out any differences between the diverse clinic forms of HD and a group of healthy subjects[10],

Taking into account these preceding studies, we have carried out this research, in order to specify the influence in *vitro* which may have suspensions of *M. leprae* on the phagocytic and lytic capacity of PMN facing *C. albicans* and *C. pseudotropicalis.*

2. MATERIAL AND METHODS

2.1 Studied subjects

Forty-five patients were studied and clinical, histopathological, bacteriological and immunological diagnosed as belonging to the Virchowian form, being under treatment in the Baldomero Sommer Hospital, General Rodriguez, Argentina. Twenty-two of them presented *Erytema nodosum hansenicum* (ENH) at the time of the study, and twenty-three had a quiescent condition. All the patients were under treatment with DDS adding the supply of thalidomide in reactional patients without bacterial, viral or fungic infections at the moment of the study and those that presented a positive lepromin test were studied as control group.

2.2 Candida albicans and Candida pseudotropicalis

C. albicans strain was cultivated at the National Hospital Baldomero Sommer laboratory. *C. pseudotropicalis* was obtained from *the* Mycology Center in the Medicine School of the University of Buenos Aires.

Both strains were cultivated in Saboureaud medium at 37º C for 24 hours and re-suspended in TC 199 (Difco Lab.) in a dosage of 1×10^6 nil. The selection of these *Candida* strains was based on the ground that *C. albicans* is destroyed by myeloperoxidase-dependent mechanisms, while *C. pseudotropicalis* is killed by myeloperoxidas eindependent mechanisms[16]

2.3 Mycobacterium leprae

Suspensions of *M. leprae* obtained from infected armadillos' livers came from the Center of Disease Control (CDC), Atlanta, Georgia, USA. No preservative substances were added when such suspensions were traited for the attainment of lepromin, and they were adjusted to a dosage of 1×10^8 bacilli in TC 199 medium.

2.4 Phagocytosis and lysis of yeasts

The technic employed was described by Giuntoli *et al*[1] . It is based on the property of PMN of adherence to the glass and on the capacity of taking Giemsa's stain common in the phagocyted but not destroyed *Candidas,* while the killed ones present a ghostly Image[14]

The leucocytes obtained by venous punction were incubated on glass slides in contact with *Candida* suspensions for an hour in moist chambers at 37º C and immediately stained with Giemsa. The following assays were made in duplicate for such a case:

a) **PMN** incubated adding *C. albicans*
b) **PMN** previously incubated for an hour with *M. leprae* suspensions and later washed and re-incubated with *C albicans.*
c) **PMN** simultaneously incubated with *M. leprae* and *C. albicans* suspensions.

The same methodology was applied for the assays with *C. pseudotropicalis.* The

number of phagocyted yeasts by 100 neutrophils was fixed in order to evaluate phagocytosis. Lysis was expressed as the percentage of killing yeasts (ghostly image) in connection with those which were phagocyted. The statistical analysis of the results was made applying the method of variance.

3. RESULTS

The results are shown in Tables 1 to 4. From the analysis of such results we come to the conclusion that no significative differences arise from the distinct studied groups, both for the phagocytosis and for the lysis of *C. albicans* and C *pseudotropicalis.* These fúnctions in the two groups of HI) patients and in the control group turn out to be similar ($p > 0.2$). The simultaneous incubation of **PMN** with yeasts and *M leprae* did not significantly change the mean values of phagocytosis and lysis of *C. albicans* and C *pseudotropicalis* in any of the studied groups (p 0.2). In the same way not significant alteration of such values after the previous incubation with M *leprae (p 0.1).*

4. DISCUSSION

The attainable results confirm that there are not alterations in the phagocytic and lytic capacity of PMN in Virchowian patients to *C. albicans* and *C. pseudotropicalis.* The presence of *M leprae* suspensions, that trigger the enzymatic mechanisms which cause the reduction of NBT according to Goihman-Yahr's experiences[13] do not exert any stimulating effect in the in *vitro* model herein studied; either previously incubating PMN with *M. leprae* or doing it simultaneously with *M leprae* and yeasts. These observations are not necessarily against those above-mentioned authors, since the increase in the reduction of NBT provoked by M *leprae* should have a non-specific feature, similar to those produced by the endo-toxins. On the other hand, our experiences were aimed at the search of specific modifications of PMN function facing yeasts on being exposed to a known antigen as *M leprae,* since that our results show that such modifications are not produced.

It may be assumed that the damage of cell-mediated immunity (CMI)[9] significantly directed to *M leprae* in lepromatous patients, does not involve functional alterations of PMN. The myeloperosidase-H2O2 haloide system and the myeloperosidase independent system are kept untouched in these cells, in opposition to the remarks made by other authors about circulating monocytes[7].

So, if there exists any compromise of the phagocytic system in HD patients we could find it out in the macrophagic cells which seem to play a relevant role in the myco-bacterial infections either from the viewpoint of processing the antigen or in its presentation to T-lymphocyte[8] . The fact that patients were under continous treatment with DDS, suggests that this drug would not exert any action on the PMN function, in opposition to the observation practised *in vitro* by some authors[18, 21] . For that it could be conjectured that the concentration of drug in blood is not enough to establish the functional alterations of **PMN** reported *in vitro* to greater concentrations.

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TABLE 1

Phagobocytes of *Candida albicans* by **PMN**

	Without M leprae	***Previous M leprae***	***Simultaneous M leprae***
Quiescent		—	—
Virchowians n=23	X = 373.7 SE=16	X = 369.4 SE=14	X = 373.0 SE=16
Reactional	—	—	—
Virchowians n=22	X = 379.2 SE=22	X = 353.36 SE=18	X = 358.9 SE=17
Healthy	—	—	—
Controls n = 15	X = 401.7 SE = 26	X = 380.3 SE = 22	X = 3833 SE = 21

$\overline{X}$: Mean value SE : Standard error of the mean n : number of cases

TABLE 2

Phagocytosis of *Candida pseudotropicalis* by **PMN**

	Without M leprae	***Previous M. leprae***	***Simultaneous M. leprae***
Quiescent	—	—	—
Virchowians n=23	X = 372.7 SE= 17	X = 355.2 SE= 18	X = 359.7 SE=17
Reactional	—	—	—
Virchowians n=22	X = 398.4 SE=21	X = 381.4 SE=23	X = 376.0 SE= 18
Healthy	—	—	—
Controls n =15	X = 384.9 SE = 15	X = 369.0 SE = 15	X = 377.8 SE = 12

$\overline{X}$: Mean value SE : Standard error of the mean n : number of cases

TABLE 3

Killing of *Condida albicans* by PMN

	Without M. leprae	***Previous M leprae***	***Simultaneous M leprae***
Quiescent Virchowians n=23	X = 35.5% SE=1.0	X = 34.2% SE=0.7	X = 34.7% SE=09
Reactional Virchowians n=22	X = 37.4% SE = 0.8	X = 35.4%6 SE = 0.8	X = 35.9% SE = 0.7
Healthy Controls n=15	X = 35.8% SE=13	X = 34.5% SE=1.0	X = 34.7% SE=1.4

X: Mean value SE : Standard error of the mean n :number of cases

TABLE 4

Killing of *Condida pseudotropicalis* by PMN

	Without M. leprae	***Previous M leprae***	***Simultaneous M. leprae***
Quiescent Virchowians n=23	$\overline{X}$ = 34.8% SE=09	$\overline{X}$ = 34.4% SE=09	$\overline{X}$ = 34.3% SE=09
Reactional Virchowians n=22	$\overline{X}$ = 37.4% SE=0.8	$\overline{X}$ = 35.4% SE=12	$\overline{X}$ = 35.9% SE=13
Healthy Controls n=15	$\overline{X}$ = 33.5% SE=0.8	$\overline{X}$ = 33.3% SE=1.1	$\overline{X}$ = 34.8% SE=13

X : Mean value SE : Standard error of the mean n : number of cases

RESUMEN — Se estudió el efecto ***in vitro*** *de* suspensiones *de* ***M. leprae*** sobre la capacidad fagocitaria y Mica de PMN frente a ***C. albicans y C. pseudotropicalis,*** en cuarenta y cinco pacientes hansenianos y en quince testigos sapos. Los resultados obtenidos demuestran que no hay diferencias significativas entre los diferentes grupos estudiados, tanto para la fagocitosis como para la fiais de levaduras. No se observaron cambios significativos en los valores medios de estas funciones con la incubación previa o simultánea de los PMN con ***M leprae y Candidas.*** Estas observaciones confirman que no hay alteradones en la bateria enzimática de los PMN en pacientes hansenianos y que la presencia de ***M. leprae*** no ejerce efectos estimuladores ***in vitro.***

Palabras clave: ***Mycobacterium leprae.*** Neutrofilos. Hanseniasis. ***Candidez albicans. Candida pseudotropicalis.***

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