sensibilidad de la dirección del centro por facilitar el desarrollo del proyecto.

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Carlos Tornero [a,*], Mari Carmen Mafe [a], Pilar Castejón [b] y Carmen Alonso [c]

[a]*Servicio de Medicina Interna, Hospital Francisco de Borja Gandía, Gandía, Valencia, Epaña*
[b]*Dermatología, Unidad de ETS, Hospital Francisco de Borja Gandía, Gandía, Valencia, España*
[c]*Servicio de Microbiología, Hospital Francisco de Borja Gandía, Gandía, Valencia, España*

*Autor para correspondencia.
Correo electrónico: tornero_car@gva.es (C. Tornero)

Determination of the invasive capacity of nonpigmented rapidly growing mycobacteria by two different in vitro assays

Determinación de la capacidad invasiva de las micobacterias no pigmentadas de crecimiento rápido mediante dos ensayos in vitro diferentes

Dear Editor:

Within the genus *Mycobacterium*, non-pigmented rapidly growing mycobacteria (NPRGM) are among the most common species of nontuberculous mycobacteria isolated in clinical mycobacteriology laboratories. Most members of this group have been described as causing human infection,[1] including nosocomial disease. The most common species implicated in these infections are *M. fortuitum*, *M. chelonae* and *M. abscessus;* in many other cases, these bacteria are not clinically significant. Other members of this group, such as *M. peregrinum* or *M. mucogenicum*, are found less often.

Despite the importance of NPRGM as human pathogens, few in vitro studies have investigated the pathogenic mechanisms of these microorganisms and the relationship between these mechanisms and virulence. One study has shown differences in growth characteristics and colony phenotype between pathogenic and non-pathogenic strains of *Mycobacterium abscessus*.[2] In another study using a strain of *M. smegmatis* as a negative control, Bermudez et al. demonstrated that *M. avium* invades HEp-2 monolayers.[3] Herein, we report the results of a study evaluating the relationships between cellular invasiveness, clinical significance, and colony phenotype of NPRGM strains isolated from human samples, together with collection strains.

The study was carried out with collection strains and clinical strains of NPRGM. The clinical strains were isolated from samples processed for mycobacterial culture in the Mycobacteriology Laboratory of Fundación Jiménez Díaz (Madrid, Spain). Strains were identified according to commonly recommended schemes, using biochemical tests and PCR-restriction enzyme analysis (PRA)[1]. The clinical significance of the strains was assessed by reviewing the patients' clinical charts according to internationally accepted criteria.[4]

For colony phenotype and fibroblast microcolony study, we followed our previously described protocol. To investigate invasion in HEp-2 monolayers, the experiment was developed modifying the method described by Bermudez et al.[3] Results were statistically analyzed using a contingency table and the chi-square test.

The 18 collection strains used in the study pertained to 16 different species, and the 74 strains obtained from clinical samples belonged to 5 different species (3 strains of *M. abscessus*, 24 *M. chelonae*, 33 *M. fortuitum*, 6 *M. mucogenicum* and 8 *M. peregrinum*). Twenty-four of these strains were considered clinically significant.

In the fibroblast microcolony assay, 35 strains presented rough colonies and only 12 strains showed elongated colonies.

All strains but one infected HEp-2 monolayers. Fifteen strains showed counts of 1 to 1000 CFU/mL and 45 strains showed counts of 1000 to 10 000 CFU/mL. Twenty-nine strains had counts of 10 000 to 100 000 CFU/mL and 2 strains, both of them *M. chelonae*, had counts of more than 100 000 CFU/mL.

The statistical analysis showed no relationship between a rough or smooth phenotype and the capacity to invade fibroblasts or HEp-2 cells. Furthermore, no relationships could be established between the clinical significance of the isolates, and the capability for intracellular penetration or the specific colony phenotype.

The presence of a rough or smooth colony phenotype in conventional agar culture has been suggested as a potential sign of pathogenicity. Several studies performed with *M. abscessus*[2,5] and *M. avium*[6] using *in vitro* and *in vivo* models have shown that strains producing rough colonies are pathogenic, whereas those producing a smooth variant are not. Nevertheless, these studies are limited by the small number of strains analyzed. In a preliminary report performed with a small number of clinical isolates, we found that fibroblast invasiveness was not related to the capability of a strain to cause human disease.[7] Although in that experiment fibroblast invasiveness seemed to appear in strains isolated from severe disease (bacteremia), in our present study performed in a large number of strains, this preliminary result was not confirmed.

We found that smooth and rough colonies appeared as often in clinically significant strains as in non-significant ones. Therefore, the phenotype does not seem to be associated with the capacity of a strain to produce human infection. No relationship was established between clinical significance and cellular invasiveness in either cell culture model. Only 5 of the 24 clinically significant strains showed elongated microcolonies; hence this characteristic does not seem to be directly related to the capability to produce human infection.

Several experiments have demonstrated that mycobacteria can also invade epithelial cells that are non-professional phagocytes, such as HEp-2 cells.[3,8] Again, our data indicate that there is no relationship between the capacity to invade HEp-2 cells and the clinical significance of the strain. Our study, using a large number of strains from several species, demonstrated that invasiveness for fibroblast and HEp-2 cells was not related to the clinical significance of the strains; therefore, this capability seems to be a pathogenic factor of minor importance in the development of human disease.

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Conflicts of interest

All authors declare no conflict of interest.

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Nieves Z. Martín-de-Hijas *, Ignacio Gadea, David García-Almeida y Jaime Esteban

Department of Clinical Microbiology, Fundación Jiménez Díaz-UTE, Madrid, Spain

*Autor para correspondencia.
Correo electrónico: bastet.sekhmet@gmail.com (N.Z. Martín-de-Hijas).

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Hipo incoercible y trastornos de conducta: una presentación atípica de leucoencefalopatía multifocal progresiva por virus de la inmunodeficiencia humana

Persistent hiccup and conduct disorder: An atypical clinical presentation of HIV-associated progressive multifocal leukoencephalopathy

Sr. Editor:

La leucoencefalopatía multifocal progresiva (LMP) es una enfermedad desmielinizante de curso progresivo y evolución rápidamente mortal que afecta a entre el 4 y el 8%[1,2] de los pacientes con sida, con una supervivencia media de 1 a 6 meses al diagnóstico (3,6 meses a 6 meses)[1,2]. A pesar de que su incidencia ha disminuido con el uso del tratamiento antirretrovírico de gran actividad (TARGA)[3], el retraso en la detección de pacientes con LMP sin diagnóstico previo de virus de la inmunodeficiencia humana (VIH) puede ensombrecer el pronóstico. Presentamos el caso de un paciente con sintomatología atípica: hipo, trastornos de la marcha y conducta extraña.

Varón de 35 años que ingresó en la unidad de agudos de psiquiatría debido a trastornos de conducta de 3 meses de evolución. Entre sus antecedentes destacaban: transfusión sanguínea hace 8 años por trombocitopenia no especificada, hábito tabáquico (20 cigarrillos por día), consumo habitual de cannabinoides y ocasional de cocaína y alcohol. Una única valoración psiquiátrica, 2 meses antes del ingreso, sin concretarse ningún diagnóstico. A su llegada a urgencias su familia relató múltiples alteraciones de la conducta: hablaba sólo con Dios, robaba macetas que regalaba a su madre, conducía su moto en ropa interior, se mostraba desinhibido y heteroagresivo hacia su pareja. Tenía conflictos legales y lo habían despedido de su trabajo. Además tenía hipo pertinaz no resuelto con clorpromacina (25 mg/8 h durante un mes), progresiva disminución de fuerza en las extremidades inferiores (deportista de alto nivel en el pasado), pérdida ponderal gradual y disfunción eréctil no resuelta con sildenafilo (100 mg/día) a demanda en el último año. La exploración neurológica evidenció, junto con un deterioro cognitivo de corte frontal (trastornos de conducta, memoria reciente alterada), signos de piramidalismo (pérdida de fuerza, hiperreflexia global y clonus bilateral) y marcha con aumento de la base de sustentación con tándem inestable. Se procedió a su ingreso en la unidad de Psiquiatría por las crecientes alteraciones conductuales que hacían claudicar a la familia. Pruebas complementarias: anticuerpos contra virus de la inmunodeficiencia humana (AcVIH) positivo, serologías de sífilis, toxoplasma, criptococos y virus de la leucemia/linfoma de células T humanas negativos. Hemograma y bioquímica: sin alteraciones. Proteinograma: normal. Poblaciones linfocíticas: linfocitos totales 1.376 µl, linfocitos CD4 14% (179 cel/µl). Carga vírica (CV): 109.000 copias/ml. Punción lumbar: glucosa 47 mg/dl, proteínas 59 mg/dl y 20 células blancas/mm^3. Reacción en cadena de la polimerasa (PCR) de virus JC (JCV) en líquido cefalorraquídeo negativo. Tomografía computarizada craneal: sin hallazgos significativos. Resonancia magnética cerebral (fig. 1): compatible con LMP. Se inició tratamiento antirretrovírico con tenofovir/emtricitabina (TDF/FTC) (200/245 mg/día) y lopinavir con ritonavir (400/100 mg/12 h), con lo que se evidenció mejoría clínica, sobre todo a nivel motor, tras 2 meses de ingreso. En posteriores revisiones persistían los fallos amnésicos, la escasa resonancia afectiva con absoluta indiferencia sobre su situación y la conducta desinhibida junto con una paresia