Original Articles


Ramanuj Lahiri, Baljit Randhawa, and James L. Krahnbuhl. Effects of Purification and Fluorescent Staining on Viability of Mycobacterium leprae .................................................................................................................. 194

Case Reports

Tarun Narang, Sunil Dogra, and Inderjeet Kaur. Borderline Tuberculoid Leprosy with Type 1 Reaction in an HIV Patient—A Phenomenon of Immune Reconstitution ................................................................. 203

Tarun Narang, Sunil Dogra, and Inderjeet Kaur. Co-localization of Pityriasis Versicolor and BT Hansen’s Disease ........................................................................................................................................... 206

Commentary

Ottenhoff, Tom H.M., and Klein, Michèl R. Leprosy Bacillus Triggers the Wrong Cells .......................................................................................................................................................... 208

Editorials

Rao, P. Narasimha. Leprosy Program in India at the Crossroads ................................................................................................................................. 211


Correspondence

Premkumar, Ramaswamy, Rajan, Pichaimuthu, and Daniel, Ebenezer. Quantitative Measurement of Sensory Impairment in Referral Centers ........................................................................................................ 219

Rada, Elsa Maria, Zambrano, Edgar A., Aranzazu, Nacarid, and Convit, Jacinto. Serologic Recognition of Low Molecular Weight Mycobacterial Protein Fractions in Lepromatous Patients with Type II Reactions (ENL) .................................................................................................................... 222

Santos, Mônica Nunes Souza, Ferreira, Luis Carlos de Lima, and Talhari, Sinésio. Paucibacillary Treatment for Large Tuberculoid Lesions of Leprosy? ............................................................................................................ 225

Kumarasinghe, S. Prasad W., and Kumarasinghe, M. P. Reply to the Editor ........................................................................................................................................ 227

Ganapati, R., and Pai, V. V. Has the Term “Elimination” Outlived Its Utility? ................................................................................................................................. 229

News and Notes

U.S.-Japan Meeting, 2004 .................................................................................................................................................................................. 230

Special Grantors ........................................................................................................................................................................................................... 238
Images from the History of Leprosy

Kalaupapa, Hawaii.
Music was an integral part of peoples’ lives at Kalaupapa, Hawaii. In 1901, the Kalawao Choir posed alongside Father Damien’s Church. This image was electronically reproduced from an original black and white photograph.
Photo courtesy of IDEA.
Clinical and Histologic Variations Among Thirty Patients with Lucio's Phenomenon and Pure and Primitive Diffuse Lepromatosis (Latapi's Lepromatosis)¹

Thomas H. Rea and Robert S. Jerskey²

ABSTRACT

The clinical and histologic experience with 30 patients who had Lucio’s phenomenon, and pure and primitive diffuse lepromatosis (Latapi’s lepromatosis) has been reviewed. The unanticipated clinical findings were a male to female ratio of nearly 1:1, a 21 month median time of onset of erythema nodosum leprosum (Type 2 reaction) after starting antibacterial treatment, and an absence of a stocking-glove pattern of anesthesia in 7 patients. The only unanticipated histologic finding was a lepromatous-granulomatous vasculitis, occurring in comparatively large vessels, or in vessels made large by pathologic changes, located near the dermal-subcutaneous interface. This finding was present in 6 of the 22 patients with histologic material available for review. In 2 of these 6 this vasculitis was identified before the onset of Lucio’s phenomenon. With one conspicuous exception, the onset of treatment with a microbicidal agent was associated with a cessation of new lesions of Lucio’s phenomenon within one week. Long-term morbidity, other than Type 2 reaction, was found in 22 of the 25 patients followed for more than 1.3 years. Usually this was the consequence of Latapi’s lepromatosis, specifically venous insufficiency and/or loss of protective sensation, and only rarely from Lucio’s phenomenon, specifically scar formation. Briefly summarized are the seven patients who had had a skin biopsy before the onset of Lucio’s phenomenon, as well as the two patients who were considered to be atypical. Criteria for the diagnosis of Latapi’s lepromatosis, in the absence of Lucio’s phenomenon, are also considered.

RÉSUMÉ

Cet article s’est attaché à faire la revue de l’expérience clinique et histopathologique de 30 patients atteints de phénomène de Lucio et/ou de lèpre lépromateuse pure et primitive-
ment diffuse, encore appelée lèpre lépromateuse de Latapi. Les données cliniques sur-
prenantes furent un ratio homme-femme de presque 1:1, un temps médian de 21 mois entre
la mise en ouvre d’un traitement antibactérien et le déclanchement d’un érythème nouveau
lépreux (réaction de type 2), et l’absence d’une anesthésie distribuée en bas-résille chez 7
patients. La lésion histologique non anticipée a été la découverte d’une vasculite léprome-
taise et granulomateuse atteignant des vaisseaux de diamètre relativement élevé ou bien des
vaisseaux élargis par les changements histopathologiques, situés près de l’interface
derme/hypoderme. Cette lésion était présente chez 6 des 22 patients qui présentaient du
matériel histologique pour une revue. Chez 2 de ces 6 individus, cette vasculite fut identifiée
avant le déclenchement du phénomène de Lucio. Lors de phénomène de Lucio et à l’excep-
tion d’un cas plutôt remarquable, l’apparition de nouvelles lésions a été interrompue dans la
semaine qui a suivi la mise en place d’un traitement avec un agent bactéricide. Une morbidi-
eté à long terme, autre que les réactions de type 2, fut trouvée chez 22 des 25 patients qui ont
été suivis pendant plus de 1,3 ans. Le plus souvent, cette morbidité était la conséquence de
la lèpre lépromateuse de Latapi, spécifiquement l’insuffisance veineuse et/ou la perte de sen-
sibilité protectrice, et seulement rarement les conséquence du phénomène de Lucio, spéci-
fiquement l’apparition de cicatrices. Brièvement résumés sont les 7 patients qui ont eu une
biopsie cutanée avant l’apparition d’un phénomène de Lucio, ainsi que les 2 patients qui
furent considérés comme atypiques. Les critères pour le diagnostic de lèpre lépromateuse de
Latapi, en l’absence de phénomène de Lucio, sont également présentés.

RESUMEN

Se hizo una revisión de los datos clínicos e histológicos de 30 pacientes que habían tenido
el fenómeno de Lucio y lepromatosis difusa pura y primitiva (lepromatosis de Lucio). Los
hallazgos clínicos no anticipados fueron: una relación masculino: femenino casi de 1:1, un
tiempo promedio de aparición de eritema nodoso leproso (reacción de tipo 2) de 21 meses
después del inicio del tratamiento antibacteriano, y ausencia del patrón de anestesia “media-
guante” (stocking-glove) en 7 pacientes. El único hallazgo histológico no anticipado fue una
vasculitis lepromatosa-granulomatosa, presente en vasos comparativamente grandes o en
vasos agradados por los cambios patológicos, localizados cerca de la interfase dermo-
subcutánea. Este hallazgo estuvo presente en 6 de 22 pacientes con material accesible para
revisión. En 2 de estos 6 pacientes la vasculitis fue identificada antes de la aparición del
fenómeno de Lucio. Con una sola excepción, el tratamiento con un agente microbicida es-
tuvo asociado con la suspensión, en la primera semana, de nuevas lesiones del fenómeno de
Lucio. La morbilidad crónica, diferente a la reacción de tipo 2, se encontró en 22 de 25 pa-
cientes seguidos por más de 1,3 años.

Usualmente la insuficiencia venosa y/o la pérdida de sensación protectora fueron conse-
cuencia de la lepromatosis de Lucio y sólo raramente del fenómeno de Lucio en cuyo caso
la consecuencia más frecuente fue la formación de cicatriz.

Se describen brevemente los casos de los 7 pacientes que habían tenido una biopsia de
piel antes de la aparición del fenómeno de Lucio, y de dos pacientes considerados como
atípicos. También se discuten los criterios para el diagnóstico de la lepromatosis de Latapi
en ausencia de fenómeno de Lucio.

Since reporting 10 patients with Lucio’s
phenomenon (25), seen in this institution
from 1969 through 1977, a further 20 had
been observed by the end of 2004. The
primary purpose of this report is to describe
the kinds and the extent of the clinical and
histologic findings in all these 30 patients
with Lucio’s phenomenon as well as in the
underlying diffuse lepromatosis. In addi-
tion, this report presents data on long-term
follow-up, and details information concern-
ing patients who had had a skin biopsy
prior to the onset of Lucio’s phenomenon.

Also, a higher incidence of a lepromatous-
granulomatous vasculitis (L-GV) was
found in patients with Lucio’s phenomenon
than was present in those with erythema no-
dosum leprosum (Type 2 reaction) or non-
reactional lepromatous leprosy.

Concerning history and nomenclature, in
1852 Lucio and Alvarado (16) reported a
necrotic skin reaction that occurred in lep-
rocy, as judged by the concomitant changes of peripheral neuropathy, eyebrow loss, and
nasal involvement. These authors also de-
scribed the absence of the nodular, dermal
lesions expected in leprosy, as well as the associated fatal termination. Latapi and Zamora (15) established that the necrosis was a result of vascular involvement, and that the absence of dermal nodules was a part of a diffuse lepromatous infiltrate, which they described in considerable detail. Also, they reported a much better prognosis with dapsone therapy. Latapi and Zamora called the necrotic skin reaction “Lucio’s phenomenon” or “erythema necroticans” and the diffuse, non-nodular lepromatous infiltration “pure and primitive diffuse lepromatosis.” For the latter expression, the synonym “Latapi’s lepromatosis” is proposed, and will be used hereafter herein. This gives an appropriate and brief eponymic recognition of Latapi’s important contributions. Also, having “Lucio” in two closely related eponymic terms, i.e., “Lucio leprosy” and “Lucio’s phenomenon,” often is a needless source of confusion.

The initial report of Lucio and Alvarado was virtually forgotten in 50 years (15). In the over 50 years following the report of Latapi and Zamora (15), both Lucio’s phenomenon and the underlying Latapi’s lepromatosis have been recognized in diverse ethnic groups, and in a wide geographic distribution, including, but not limited to, Louisiana (8), Hawaii (3), Brazil (1, 9, 31), Greece (11), the Near East (30), India (29), Singapore (2), Indonesia (13), and Polynesia (6). Apparently the condition remains rare except in Mexicans, Costa Ricans (28), and Cubans (18), where its incidence is aptly described as “not common.”

This retrospective study is somewhat incomplete because of the institutional policy of “deep” storage of charts, and the Northridge earthquake of January 1994 trashed the room holding both histologic glass slides and paraffin blocks. The material available was considered sufficient to illustrate a range of clinical and histologic findings, the responses to treatment, and the long-term morbidity encountered.

**MATERIALS AND METHODS**

The subjects were patients in the Hansen’s Disease Clinic or the Dermatology Clinic of the Los Angeles County-University of Southern California Medical Center. Included in this study were all patients who had one or more characteristic lesions of Lucio’s phenomenon, i.e., serrated hemorrhagic infarcts usually arising in crops, and at least one characteristic sign of Latapi’s lepromatosis (see below) or of lepromatous leprosy, but no lepromatous nodules. The diagnosis of Latapi’s lepromatosis was made after the fact of Lucio’s phenomenon. No criteria for exclusion were established.

The data base for the clinical information in this study was compiled from five sources: the available charts, data abstracted from charts for previous publications, clinical photographs obtained before starting treatment, patients currently being followed in clinic, and available histologic specimens. The summary of the histologic changes was compiled from the material available for review.

**RESULTS**

The results will be presented in two ways. First will be 9 brief case reports. Following the case reports, the available data on all patients will be summarized in narrative form.

**CASE REPORTS**

The initial 7 case reports concern those patients who had had skin biopsies taken prior to the development of Lucio’s phenomenon. Five of these biopsy specimens had been seen by one of us (THR), and 3 of these 5 were available for review. Among these 7 patients, 4 had a diagnosis of leprosy established and treatment initiated before the onset of Lucio’s phenomenon (Cases 2, 3, 6, and 7), whereas 3 had had a biopsy but the diagnosis of leprosy was not made until the onset of Lucio’s phenomenon (Cases 1, 4, and 5). The remaining 2 case reports (Cases 8 and 9) concern those who were considered to be atypical.

**Patients with skin biopsies prior to Lucio’s phenomenon**

Case 1. An 18 year old woman sought care because of numbness of the hands and feet of several months duration. Neurologists interpreted her findings to be a peripheral neuropathy secondary to a systemic disease. Consultation with many medical specialties could identify no systemic illness. After 18 months, a skin biopsy taken from an area of diminished sensory perception, but otherwise clinically normal skin,
<table>
<thead>
<tr>
<th>Table 1</th>
<th>Frequency of clinical signs of Latapi’s lepromatosis.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of patients evaluated</td>
</tr>
<tr>
<td>I. Clinical <em>sine qua none</em> for Latapi’s Lepromatosis</td>
<td></td>
</tr>
<tr>
<td>A. Diffuse non-nodular Lepromatous leprosy</td>
<td>30</td>
</tr>
<tr>
<td>II. Highly suggestive of Latapi’s lepromatosis</td>
<td></td>
</tr>
<tr>
<td>A. Telangiectasias</td>
<td>28</td>
</tr>
<tr>
<td>1. eruptive</td>
<td>3</td>
</tr>
<tr>
<td>2. mats on face and trunk</td>
<td>7</td>
</tr>
<tr>
<td>B. Not visible subcutaneous plaques</td>
<td>28</td>
</tr>
<tr>
<td>C. Signs of diffuse infiltration</td>
<td>28</td>
</tr>
<tr>
<td>1. non-marginated induration of cheeks</td>
<td>4</td>
</tr>
<tr>
<td>2. widening of nasal root</td>
<td>9</td>
</tr>
<tr>
<td>3. swollen backs of hands</td>
<td>9</td>
</tr>
<tr>
<td>4. dusky swelling of feet</td>
<td>1</td>
</tr>
<tr>
<td>III. Consistent with Latapi’s lepromatosis</td>
<td></td>
</tr>
<tr>
<td>A. Alopecia of eyebrows</td>
<td>30</td>
</tr>
<tr>
<td>1. total</td>
<td>20 (67)</td>
</tr>
<tr>
<td>2. partial</td>
<td>7 (23)</td>
</tr>
<tr>
<td>B. Rhinitis</td>
<td>30</td>
</tr>
<tr>
<td>C. Septal perforation</td>
<td>25</td>
</tr>
<tr>
<td>D. S-GPSI with little motor change</td>
<td>30</td>
</tr>
</tbody>
</table>
was interpreted as “normal skin.” Three years after her initial presentation crops of infarcts of Lucio’s phenomenon quickly led to the correct diagnosis. A Fite stain then done on the “normal skin” material demonstrated acid-fast bacilli (AFB) and globi within nerves and endothelial cells (slide not available for review).

Comment: this case illustrates the difficulty of making a diagnosis of leprosy in the absence of a sign more readily associated with leprosy.

Case 2. A 22 year old woman presented to the obstetrical service in labor with an incidental complaint of eyebrow alopecia, progressing from medial to lateral, of 2 months duration. Because rhinitis was also present, leprosy was considered. A biopsy of clinically normal skin showed AFB and globi in some endothelial cells and perivascular macrophages (slide not available for review). No AFB were found in placental endothelium. One month after initiating dapsone monotherapy, erythema nodosum leprosum (ENL) developed, which was managed with thalidomide. Eight months later the patient was lost to follow-up, only to return after a 13 months absence with the necrotic lesions of Lucio’s phenomenon and ulcers (biopsy not available for review).

Comment: This case confirms the possibility of considering a diagnosis of Latapi’s lepromatosis without the infarcts of Lucio’s phenomenon, as suggested by others (9). Also, this is the only patient seen in this series who had ENL prior to having Lucio’s phenomenon. Because of the absence of AFB in placental endothelial cells, but their abundance in cutaneous endothelium, the predilection of AFB for endothelial cells is likely to be organ specific.

Case 3. A 45 year old woman presented with eyebrow alopecia, rhinitis, and palpable but not visible, indurated lesions in the subcutis. Skin biopsy showed, in addition to an extensive lepromatous infiltrate, an AFB-positive, lepromatous-granulomatous vasculitis (L-GV) in the subcutis, characterized by endothelial proliferation, macrophages dissecting between the smooth muscle cells of the media, and macrophages infiltrating the adventitia (Fig. 1a, b). Also present was an aneurysmal lepromatous-granulomatous out-pouching originating in a subcuticular vessel (Fig. 1c), as well as signs of new vessel formation. Our working diagnoses were lepromatous leprosy and a reactional state of unknown type. She was treated with daily dapsone and rifampin, as well as prednisone and thalidomide. The unknown reactional state was managed with decreasing doses of thalidomide and prednisone, the latter being discontinued after 5 months. Seven months after initiating treatment, and 2 months after stopping prednisone, but still taking thalidomide 100 mg daily, the patient developed Lucio’s phenomenon (Fig. 1d). Her new Lucio infarcts ceased within 2 weeks upon resuming prednisone at 40 mg daily; the dose being tapered slowly over the ensuing 5 months.

Comment: Two explanations for the sug-
FIG. 1. From Case 3. a) A low power view of a biopsy from a palpable, but not visible, subcutaneous lesion, on the right arm, which shows in the lower right corner an enlarged vessel in the subcutis with a conspicuous internal elastic lamina. The lumen is occluded, and adventitial involvement is apparent. Heavy infiltration of the dermis is evident. 1.25× objective. b) A high power view of the same vessel as shown in “a.” The now less conspicuous internal elastic lamina is indicated by wide arrows, but is readily seen in color. The lumen is occluded.
suggested “erythema nodosum” appear possible. A leprologic hypothesis is the development of mild, neutrophil-free, transient ENL in a patient who had yet to express other clinical manifestations of leprosy. An alternative explanation is that of a leprosy-unrelated inflammatory disorder occurring on a lepromatous background.

**Case 5.** A 28 year old man sought care because of the development of palpable, but not visible, nodules in the subcutaneous tissue of his legs. The clinical impression was a vasculitis. A skin biopsy was interpreted as “nodular vasculitis,” the pattern consisting of endothelial proliferation with occlusion of the lumen, as well as a dense infiltrate of macrophages in the adventitia (Fig. 3a). He was begun on an anti-inflammatory regimen, which included methotrexate and prednisolone. Five months later he developed an acute, febrile illness, diagnosed as a Salmonella non-typhoid bacteremia, secondary to the ingestion of snake powders. He responded well to intravenous ciprofloxacin, and was discharged on ciprofloxacin 500 mg twice daily orally, methotrexate 5 mg twice daily, and prednisone 80 mg every other day in the morning. One day after discharge he abruptly developed extensive and widely distributed infarcts of Lucio’s phenomenon. These involved about one-third of his body surface area, most extensive on the legs (Fig. 3b), and arms, but present also on his ears, face (Fig. 3c), trunk, scrotum, and the urethral meatus. This patient was promptly readmitted, and 1 day later his right hand was affected by apparently complete arterial occlusion. Circulation was restored by prompt intervention, but deep necrosis eventually resulted in the loss of the 5th right distal metacarpal head and the right 5th finger. His left patella was also lost, as a consequence of extensive tissue necrosis over the left knee. A Fite stain on the tissue initially interpreted as “nodular vasculitis” was positive for AFB in endothelial cells and adventitial macrophages.

**Comment:** This one patient recapitulates three prior case reports. 1) Leprosy may mimic nodular vasculitis (34). 2) Occlusion of large muscular arteries may occur in association with Lucio’s phenomenon (8). 3) Lucio’s phenomenon may occur in a setting of convalescence from serious infection, i.e., in 2 cases of erysipelas managed with penicillin (1). (These 2 patients, and Case 5, received antibiotics ineffective against *M. leprae.* New infarcts ceased when rifampin was begun. This patient was the only one whose prognosis was poor when first seen.

**Case 6.** A 37 year old woman presented to another clinic because of eyebrow alopecia and rhinitis. A skin biopsy confirmed the impression of lepromatous leprosy (slide not available for review). She took 100 mg of dapsone daily for 18 years. At age 68, 13 years after stopping dapsone, she presented to this clinic with Lucio’s phenomenon, occurring intermittently for one month.

**Case 7.** A 15 year old boy presented to a dermatology clinic in Mexico City because of eruptive telangiectasias and eyebrow alopecia. A skin biopsy (not available for review) established a diagnosis of leprosy. Dapsone treatment was initiated at that time but he took it infrequently. At age 21 he presented to our clinic because of leg ulcers and skin infarcts of 6 months duration; telangiectasias were prominent at that time. Dapsone was resumed, but he was seen infrequently. At age 35 he made 2 visits to our clinic because of leg ulcers. Two skin biopsies performed at that time were negative for AFB (not available for review).

**The atypical patients**

**Case 8.** This 45 year old woman satisfied entry criteria for this study on clinical grounds because she had one 8 mm characteristic infarct, a perforated nasal septum, total alopecia of eye lashes and eye lids,
FIG. 2. From Case 4. a) From a Lucio’s phenomenon lesion, a vessel in the subcutis with prominent endothelial proliferation, and adventitial infiltration. 40× objective. b) A low power view of an earlier biopsy obtained from a red dermal nodule 4 years before the onset of Lucio’s phenomenon, showing epidermal thickening, a superficial infiltrate in the upper dermis, and an arcuate infiltrate in the mid dermis. 10× objective. c) An oil-immersion view from the arcuate infiltrate showing 2 nucleated Virchow cells and the cytoplasm of a 3rd. Lymphocytes are evident, but no neutrophils were found. 90× objective.
several stellate scars, and 3 large leg ulcers, each approximately 10 cm in diameter. Biopsy showed a heavy infiltrate of macrophages, AFB and globi in some endothelial cells, but no endothelial proliferation. She is considered to be atypical because of a “peau d’orange” appearance to the skin of her forehead and cheeks, as well as beading on corneal nerves (15). Also, she is the only patient in this series with just one infarct, and one of two without endothelial proliferation.

Comment: At present it is unknown if any of these atypical findings, or a combination thereof, would constitute valid criteria for exclusion.

Case 9. A 24 year old man presented to this clinic with typical Lucio’s phenomenon, and giving a history of similar clinical findings, as well as intermittent dapsone use for 4 years. A lesional biopsy did not show epidermal necrosis (probably exfoliated during processing), or congestion of superficial vessels, or extravasation of erythrocytes, but did demonstrate AFB in proliferating as well as in non-proliferating endothelial cells, globi in endothelial cells, and passive congestion of deep dermal vessels. Also present were larger vessels with lumens occluded from endothelial proliferation, and macrophage infiltration of the adventitia, but no infiltration of the media (Fig. 4a). New lesions ceased to form 4 weeks after starting dapsone monotherapy. To this point, on balance, the patient was considered to be typical. Eight years later, in the setting of seemingly good compliance with dapsone monotherapy, new Lucio’s phenomenon-like infarcts developed on the trunk and extremities. With the working diagnosis of a relapsing Lucio’s phenomenon, and an inference of dapsone resistance, dapsone was discontinued, being replaced by daily rifampin and daily minocycline, and the new lesions ceased after 8 weeks. Biopsy now showed epidermal necrosis, passive congestion of superficial vessels, and endothelial proliferation in deep dermal vessels, but stains for AFB were repeatedly negative (slides not available for review).

Comment: No support for dapsone resistance was found. A self-limited process of unknown type, unrelated to leprosy, but mimicking Lucio’s phenomenon must be considered.

### SUMMARY OF ALL CASES

#### Demographic data

Sixteen men and 14 women satisfied inclusion criteria. Twenty-five were born in Mexico, 2 in Cuba and 3 in the United States, each of the latter 3 also having resided in known leprosy-endemic areas of Mexico for more than 5 years. Age at the time of diagnosis of Lucio phenomenon, leprosy was known in 29 and ranged in duration from 15 to 71 years, with a median of 34 and an average of 33.7 years.

#### First sign or symptom of leprosy, and time to diagnosis

The first sign or symptom attributed to leprosy was alopecia in 16 (eyebrows in 15, extremities in 1), leg ulcers in 4, sensory impairment in the hands and/or feet in 3, nasal symptoms in 2 (1 with nose bleeds, 1 with congestion), eruptive telangiectasias in 2, and infarcts of Lucio’s phenomenon in 2, but was not recorded in 1. The elapsed time from the initial sign or symptom to the diagnosis of leprosy ranged from a minimum of 2 months to a maximum of 10 years, with a median of 3 years and an average of 4.1 years.

#### Mode of presentation

Of the 28 patients presenting to our clinic with Lucio’s phenomenon, the presenting complaint was leg ulcers in 14 (duration 2 months to 7 years, median 8 months and average 19.7 months). The other 14 complained of the infarcts of Lucio’s phenomenon, usually occurring in intermittent crops (duration 5 days to 10 years, median 4 months, and average 4.1 months). Both infarcts and ulcers were characteristically present at presentation, excepting Case 5, who had no ulcers at presentation. The infarcts were hemorrhagic, slightly indurated, not tender, but sometimes painful. Sharply marginated, irregularly serrated borders were characteristic, such that an observer viewing the infarct, whether large or small, from “normal” skin would see a concavity (Fig. 3b, c). Early, pre-hemorrhagic lesions were seen in only one patient, being slightly indurated, light blue in color, and having an erythematous halo. (Previously published photomicrographs of this specimen are figs. 4 and 5 in Rea and Levan (25), and figs. 3 and 4 in Quismorio, et al. (23)) The least
number of infarcts recorded was one, in Case 8. In all other patients the lesions were multiple, arising in crops, most commonly on the legs, but less frequently on the thighs and forearms. In one patient, Case 5, the infarcts were numerous (over 100), widely distributed, and clearly placed his life in peril. In another patient, 24 infarcts were counted. And in yet another patient the infarcts were small, and present only below the ankles. Infarcts on the arms resolved rapidly, behaving more like erosions than ulcers. In 2 patients the infarcts became bullous. Infarctions in organs other than the skin were not evident.

Ulcers were common on the legs, but occasionally on the thighs. Ulcers of recent onset appeared to be the direct sequelae of the infarcts, being ovoid and irregular in shape, not exceeding 5 cm in greatest diameter. If of long standing, ulcers were round in shape and up to 10 cm in diameter, perhaps being complicated by neglect and unsuitable topical therapy.

In two patients the onset of Lucio’s phenomenon was associated with pregnancy, each ending with a normal delivery. In Case 5, the onset occurred while the patient was convalescing from a Salmonella bacteremia. No other potentially precipitating events could be identified.

Signs of Latapi’s lepromatosis at the time of presentation

Absent nodules. The absence of lepromatous dermal nodules was noted in all 30 patients, with the possible exception of Case 8, who had a “peau d’orange” prominence to the follicular orifices on the face, presumably the result of infiltration or edema, but no dermal nodules in the conventional sense.

Diffuse infiltration. Signs of diffuse cutaneous infiltration were not mentioned as either present or absent in 8 patients. One or more signs of diffuse infiltration were recorded as present in 22 patients. Widening of the nasal root was described in 9 (or retrievable from clinical photographs). Diffuse infiltration in the hands was noted in 9 patients, being variously described as “swelling,” or “non-pitting edema,” of the backs of the hands, in association with “fusiform fingers.” Changes in the cheeks of the face were variously described in 4 as “red plaques, poorly marginated,” “indurated erythema,” or “a cyanotic edema.” “Full” or “swollen” ear lobes were stated to be present in 7 patients. A “dusky swelling” of the feet was described in 1. (Swollen ear lobes and fusiform fingers are signs of diffuse infiltration not commonly found in patients with ordinary lepromatous leprosy.)

Subcutaneous plaques. Not visible, nontender, subcutaneous plaques were found on the arms or legs in 8 patients, but were not mentioned as absent in any of the 20 other charts available for review.

Telangiectasias. Telangiectasias were described as eruptive in 3 patients; these persisted in 2, but treatment-associated remission occurred in 1. Persistent telangiectatic mats, occurring on the face or upper chest, were noted as present in 7, (masquerading as spider angiomas, but having no central arteriole, and upon expression, filling from the periphery).

Ordinary changes of lepromatous leprosy at time of presentation

Alopecia. Alopecia of the eyebrows was stated to be complete in 20, partial in 7, and was not noted as present or absent in 3. Rhinitis. Rhinitis was stated to be present in 25, and was not mentioned as present or absent in 5. Of the 25 with recognized rhinitis, the nasal septum was recorded as perforated in 9, as intact in 8, but was not mentioned as perforated or intact in 8. Stocking-glove pattern sensory impairment (S-GPSI). S-GPSI is a withering away of the sensations mediated by the type C sensory fibers, beginning distally and proceeding proximally. S-GPSI was recorded as present to some degree in 20, as absent in 7, not mentioned as present or absent in 3. Motor impairment. Motor impairment was present in only three: two patients with ulnar nerve involvement and one with common peroneal nerve involvement. The motor changes were disproportionately few compared with the magnitude of the sensory loss.

Routine laboratory findings at presentation

A mild anemia, normochromic and normocytic, was common. The average leukocyte count (normal range 3.7–11.6 × 1000/mm³) was 6.5 and the median was 6.3, among the 23 initial counts available for review. The highest count, 11.9, was
FIG. 3. From Case 5. a) In the center of the field is the largest of several vessels showing endothelial proliferation in the specimen from a palpable, but not visible, subcutaneous lesion obtained from the left thigh, 5 months before the onset of Lucio’s phenomenon. 20× objective. b) Several lesions of various sizes on the face. Most show the characteristic serrated margins. c) The left knee with adjoining thigh and leg. The skin of the anterior portion is largely necrotic, but the serrations still evident, although farther apart than in “4b.” Smaller lesions are evident posteriorly.
seen was in Case 5; the lowest was 3.1. Two counts were above and 2 were below the normal limits. Among 19 patients the cardiolipin-based serologic test for syphilis was reactive or weakly reactive in 15 in 3; of the 15 who were reactive, a Treponema-specific test was positive in 3. Hyperglobulinemia was common; in 18 patients the mean value was 4.8 gm/dl (normal 3.0–4.0), the median 5, the high 6.0 and the low 3.2. Serum albumin values were normal in 7, ranging from 3.8 to 4.6 gm/dl, clearly low in 3, ranging from 2.9 to 3.1, and extreme in 1, being 1.8 in the one patient with extensive infarcts, Case 5.

Response to treatment

_Cessation of new infarcts._ Of the 28 patients who presented to the clinic with Lucio’s phenomenon, 19 were begun on dapsone alone. Ten of these 19 had no new infarcts after one week of follow-up. The remaining 9 continued to develop new infarcts for up to 5 months after starting treatment, 2 of which appeared to worsen before they improved. In one of the latter, new lesions ceased at 6 weeks, in association with the addition of daily rifampin.

Of the 7 previously untreated patients with Lucio’s phenomenon who were started on a daily microbicidal agent (5 with rifampin, 1 with clarithromycin and 1 with minocycline) no new infarcts were seen after 7 days. (In these 7, a second daily agent was added within 2 weeks, so that all were receiving daily rifampin and daily clarithromycin or minocycline or dapsone.)

In two patients, followed for less than 4 weeks, no judgement was made as to treatment response.

Of the two patients who developed Lucio’s phenomenon after initiating anti-microbial treatment in our clinic, the one, Case 2, who developed the reaction after discontinuing dapsone, responded without new lesions after resumption of dapsone. The other, Case 3, who developed Lucio’s phenomenon reaction after 7 months of daily dapsone and rifampin is a glaring exception to the usually favorable outcome of microbicidal treatment. Her response to increased daily doses of prednisone was good, with new lesions ceasing within 2 weeks, and no recurrences in association with a slow tapering of the prednisone over 5 months.

_Healing of ulcers._ The ulcers usually healed within 4 months, in 3 patients taking as long as 8 months, _the length of time being roughly proportional to ulcer size._

Follow up data

As of December 31, 2004, 14 patients were still being followed. Of the remainder, 15 had been lost, and 1 was deceased after 20 years of follow up. The length of follow up has been as brief as less than 1 month and as long as 35 years, mean 12.9 and median 10. Five were followed for 1.3 years or less.

_Erythema nodosum leprosum_

Thirteen of the 30 patients, 7 women and 6 men, were known to have developed ENL. In Case 2 ENL definitely preceded the onset of Lucio’s phenomenon. Also Case 4, who might have had mild ENL 4 years prior to developing Lucio’s phenomenon, did develop typical ENL 37 months after initiation of treatment with daily rifampin and dapsone. Excepting Case 2, the time of onset of the ENL ranged from 1 to 41 months after treatment was started in our clinic, median 21 months, average 22.1. Apart from this long median time of onset after initiation of treatment, the ENL in these patients did not differ from the ENL seen in ordinary lepromatous leprosy. No patient had lesions of ENL at the time of having new lesions of Lucio’s phenomenon.

_Long term morbidity_

Excluding from analysis the 5 patients followed for 1.3 years or less, some degree of long-term morbidity has been experienced by 22 of the remaining 25 patients. Apart from ENL, the long-term morbidity observed in these patients arose from three mechanisms. Two of these mechanisms, S-GPSI and venous insufficiency, appear to be a part of Latapi’s lepromatosis. The third mechanism producing long-term morbidity, scar formation, was the direct consequence of Lucio’s phenomenon. Secondary to S-GPSI, 11 have experienced ongoing problems with pathologic plantar callosities and/or ulcers. Also secondary to S-GPSI, 5 have physical impairment in the hands, including fissures, ulcerations, and bone resorption. Morbidity from muscle weakness
or contracture was rare, occurring only in Case 5, and consisted of soft tissue contracture of the fascia and tendinous muscle in the left knee complex. Recurrent leg ulcers from venous insufficiency have been an ongoing problem for 10; 4 of these also having trophic problems in their feet. Scar formation led to hand and leg disabilities in one patient, Case 5.

Of the 14 who continue to be followed, 7 have a disability grade 1 or 2 according to the WHO grading system; 3 with a grade 1 disability and 4 with a grade 2 disability. Those with grade 2 disability include one with bilateral finger resorption, one with unilateral finger resorption, one with a resolving plantar ulceration, and one (Case 5) with right hand partial amputation due to ischemic changes.

Histologic changes

Histologic material obtained from lesions of Lucio’s phenomenon was available for review in 22 patients. In 15 both H&E and adequately preserved Fite stained material was available, in 5 H&E only, and in 2 adequately preserved Fite stained tissue only. The common source of variation among specimens was the age of the lesion sampled. Most of the features have been described in detail and illustrated elsewhere (25, 26).

In all 22 patients the histologic material demonstrated foamy macrophages in the dermis in association with a few lymphocytes. The volume of the dermis occupied by the macrophages was small in 16 specimens, ranging from 2–10%, with a median value of 5%. In the remaining 6, the volume occupied was larger, ranging from 15–40%, with a median of 23%. In contrast, in biopsy specimens obtained from the indurated, but not visible subcutaneous plaques present before the Lucio phenomenon in Cases 3 and 5, the volume of the infiltrate occupied approximately 70 and 80% of the dermis, respectively. In their Lucio’s phenomenon lesions the volume of the dermis occupied by macrophages was approximately 5–10% in both patients.

Epidermal necrosis was present in 18 and absent in 2 but could not be evaluated in 2 because of absent or insufficient epidermis. In the recent lesions, the necrotic epidermis was of normal thickness but manifested the absence of staining of nuclei, nuclear ghosts, and early regeneration at the periphery, which was an epithelial tongue dissecting between the necrotic epidermis and the dermis. In older lesions, a new keratinizing epidermis was well developed, the necrotic epidermis now located above the new stratum corneum, and identifiable by compacted nuclear ghosts in the former prickle cell layer and melanin in the former basal cell layer (Fig. 1d). Similarly, necrosis of eccrine ducts and/or coils was present to some degree in 16 and absent in 6. In the oldest lesions, the necrotic epidermis was evidently exfoliated in the processing, and necrotic eccrine structures had been absorbed.

Intense passive congestion of vessels was present in 16 and absent in 4, but could not be evaluated in 2. This was usually confined to the superficial dermis, but was present in the deep dermis or subcutis in 3. Extravasation of erythrocytes was present in 12 and absent in 8, but could not be evaluated in 2.

In the medium sized vessels of the dermis and subcutis, endothelial proliferation was identified in 20, but could not present in 2. The proliferation ranged from mild to severe, frequently producing luminal occlusion, and was associated with thrombosis in 7. Inflammatory cells with in these vessels were few in number, suggesting that the term “vasculosis” would be better than “vasculitis.” The generally sparse inflammatory infiltrate was primarily lymphocytic. Neutrophils or neutrophilic dust were identified in 6 of 22 specimens, but were not associated with vascular changes, but instead were infiltrating necrotic areas in 5, and in the subcutis in 1. Fibrinoid change was present in 1 specimen.

Subcutaneous tissue was present in specimens from 18 patients. The area occupied by the subcutis was estimated to be 20% or less than that of the dermis in 5, 20–50% of that of the dermis in 6, and 50% or more of that of the dermis in 7, respectively. All 18 specimens had some degree of a lobular panniculitis. This was considered to be focal in 9, involving an estimated 15% or less of the panniculus, moderate in 5, involving 20–40%, and extensive in 4, involving 50–80%. The nature of the infiltrate varied considerably, 8 with lepromatous macrophages and only a few lymphocytes, 9 with an ob-
vious mixture of leprotic macrophages and lymphocytes, and 1 with leprotic macrophages and neutrophils, the latter infiltrating between the lipocytes, apparently ignoring the vessels.

Concerning specimens with adequately preserved Fite stains, AFB and globi were found in macrophages in the dermis in all 17 specimens, as well as in the macrophages in the subcutis of the 15 specimens so endowed. In all 15 specimens with both endothelial proliferation and a Fite stain, bacilli in some of the proliferating endothelial cells were readily identified in all but 1, often with globi. Similarly, in all 17 specimens with a Fite stain, bacilli could be found in some non-proliferating endothelial cells. Bacilli were most difficult to find in endothelial cells in Case 3, probably the consequence of 7 months of continuous antimicrobial treatment.

Histologic changes in large vessels, or vessels made large by pathologic changes, which we have chosen to call L-GV, were present in a total of 6 of the 22 (27%) patients with Lucio’s phenomenon. In 2, Cases 3 and 5, the large vessel changes were observed only in non-necrotic specimens, obtained before the onset of Lucio’s phenomenon. The fully developed L-GV consisted of endothelial proliferation, macrophages infiltrating the media, and macrophages infiltrating the adventitia. This fully developed change was found in Case 3, (Fig. 1a–c), and in lesions from 2 other Lucio’s phenomenon patients. In one of these latter 2, the changes were active, (Fig. 4b, and in the other the changes were considered to be regressing (Fig. 4c). Incomplete expression of the L-GV consisted of endothelial proliferation with a variable degree of adventitial infiltration, as exemplified by Fig. 4a, also found in Figs. 2a and 3a. A similar L-GV was found in 6 of 70 (9%) of histologic specimens obtained from lesions of ENL (Fig. 4d), and as an incipient change in 3 of 51 (6%) non-reactional lepromatous patients (data not shown). These incipient changes were found in comparatively large subcutaneous vessels located near the dermis, and consisted of foci of endothelial proliferation in which AFB were identified, whereas none were found in non-proliferating endothelium. In Lucio-Latapi disease, ENL, and non-reactional lepromatous leprosy, the vessels involved with L-GV were located in the subcutis, or what was subcutis prior to lepromatous infiltration or connective tissue proliferation. The L-GV involved vessels were largest in the Lucio-Latapi patients, smallest in the non-reactional lepromatous material, and of intermediate size in specimens of ENL.

**DISCUSSION**

The findings in the additional 20 patients with Lucio’s phenomenon are in good accord with the initial report of 10 such patients (29) from this clinic. Hence the 30 patients have been taken together in this report. The additional 20 patients and the follow-up information add detail to the variations in the clinical picture without altering its broad outlines. The one exception to this accord is the finding of large vessel involvement in the subcutis, where it was not found in a previous report (29). This failure was not due to a lack of looking, but was probably the result of inadequate sampling of the subcutis with the 4mm in diameter punch biopsy instruments, then in routine use. This contrasts to the more generous amounts, and greater depths, obtained with the 6mm punches, in common use in our clinic for the past 2 decades, as exemplified by Fig. 1a.

What is being called L-GV is not a new pattern. For example, this pattern has been previously observed in non-reactional lepromatous specimens (7), as well as in specimens of ENL (17, 27). Also it has been observed and illustrated in Lucio’s phenomenon (10), and was alluded to by Latapi and Zamora (15). The pattern is similar to, if not the same as, that of the “lepromatous phlebitis” reported by Mukherjee and his associates (19, 20).

L-GV occurs in larger vessels, or in vessels made large by pathologic changes, primarily in the subcutis. L-GV, and what is interpreted as its variants, was present in 4 of the specimens from 22 Lucio’s phenomenon patients with material available for review, and in 2 of the 3 pre-Lucio’s phenomenon specimens available for review. However, the importance of L-GV to the pathogenesis of Lucio infarcts, if any, is not known.

An argument can be made to support the hypothesis that the L-GV is not important
<table>
<thead>
<tr>
<th>Case</th>
<th>Area of subcutaneous tissue expressed as % of dermis</th>
<th>% of subcutaneous tissue infiltrated</th>
<th>Lepromatous-granulomatous vasculitis</th>
<th>Epidermal necrosis</th>
<th>Intense passive congestion</th>
<th>Endothelial proliferation</th>
<th>Endothelial proliferation with lumen occlusion</th>
<th>PMN infiltrating lesion</th>
<th>Case # in text</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>&gt;50</td>
<td>75</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>&gt;50</td>
<td>15</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>&gt;50</td>
<td>40</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>9</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>&lt;20</td>
<td>60</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>20–50</td>
<td>5</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>&gt;50</td>
<td>30</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>4</td>
</tr>
<tr>
<td>H</td>
<td>20–50</td>
<td>5</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>8</td>
</tr>
<tr>
<td>J</td>
<td>20–50</td>
<td>30</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>&lt;20</td>
<td>10</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>L</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>0</td>
<td>NA</td>
<td>NA</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20–50</td>
<td>5</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>5</td>
</tr>
<tr>
<td>O</td>
<td>&lt;20</td>
<td>5</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Major histologic findings in 15 Lucio's phenomenon lesions, with both H&E and adequately preserved Fite stained material, with special emphasis on subcutaneous changes.
From Case 9, a Lucio’s phenomenon lesion. An obliquely sectioned, subcutaneous vessel showing extensive endothelial proliferation, and heavy adventitial infiltration, but little disturbance in the smooth muscle bundles. 20× objective. b) A panvasculitic vessel from the subcutis of a Lucio’s phenomenon lesion showing intimal proliferation, a chaotic infiltration of macrophages among the smooth muscle bundles, and infiltration in the adventitia. c) A high power view of a large subcutaneous vessel which shows modest endothelial change,
to the pathogenesis of Lucio’s phenomenon. Because the pattern of L-GV also has been seen in lesions of ENL (17, 27) (Fig. 4d), as well as in non-reactional lepromatous leprosy (16), and because it has similarities to the leprous phlebitis reported by others (19, 20) it is not specific for either Latapi’s lepromatosis or the Lucio reaction. Also, because its presence in Cases 3 and 5 were not directly associated with necrotic lesions, it is not a change necessarily leading to necrosis. In this context L-GV is most easily interpreted as part of the vascular changes known to be associated with leprosy, from large muscular arteries, to arterioles, to capillaries, to venules, and on to large veins. Such changes have been reported by, among others, Fite (12), Coruh and McDougall (17), Kaur, et al. (14), Bansai, et al. (5), and Mukherjee and his associates (19, 20).

An argument can be made to support the contrary hypothesis of importance for L-GV in pathogenesis. Anoxia is a final cause of tissue necrosis, however diverse the responsible mechanisms. In patients with Lucio-Latapi disease, anatomical changes with the potential of leading to anoxia have been demonstrated at three levels of the vascular tree. Best described is the endothelial proliferation with lumen occlusion, with or without thrombosis formation, occurring in the mid-sized vessels in the dermis of the necrotic lesions (25, 26, 31), as confirmed in the present study. Also, swelling and parasitization, with lumen occlusion, of capillary endothelium has been found by electron microscopy in 3 of 3 specimens of clinically normal skin (33), i.e., Latapi’s lepromatosis, from patients with Lucio’s phenomenon, and could well be widespread. The subcuticular L-GV found in the present study in both Lucio’s phenomenon and Latapi’s lepromatosis is a third anatomical change that could contribute to anoxia; this change, if focal, could also be more prevalent than has been observed. All of these changes acting synchronously might result in an ischemia sufficient to produce necrosis, whereas any one by itself would be less likely do so. In addition, the circulating immune complexes associated with Lucio’s phenomenon (23) might interact with the anatomical changes in ways that lead to necrosis.

Perhaps the changes of the L-GV are better developed Latapi’s lepromatosis, because this difficult to diagnose lepromatous condition may give more time for bacterial proliferation and for a granulomatous vasculitis to develop.

Three retrospective findings were not anticipated, but emerged only when the comparatively large numbers of patients reported here were gathered together. 1) The near parity of the genders is in contrast to the expected male preponderance, 2 to 1, in lepromatous disease (21). 2) The absence of S-GPSI in 7 patients was not anticipated to be this common in Latapi’s lepromatosis. 3) The median time of onset of ENL, 21 months after initiating treatment, appears to differ considerably, and perhaps significantly, from the 12 months median time observed in this clinic (unpublished data). Good explanations for these three unexpected findings are not available.

Clinically, the ischemic infarcts of Lucio’s phenomenon were of a uniform character, varying in size and extent, but always of the same kind, or, in other words, a monomorphic response. Accompanying ulcers, erosions and bullae, were clearly secondary to the infarct.

The report by Diogenes, et al. (8) and our experience with Case 2, raises the possibility of the diagnosis of Latapi’s lepromatosis in the absence of Lucio’s phenomenon. Usually a diagnosis of Latapi’s lepromatosis is made after the fact of Lucio’s phenomenon. Which findings or combination of findings might be considered as criteria for a diagnosis of Latapi’s lepromatosis in the absence of Lucio’s phenomenon?

Either of two findings could be regarded as a sine qua none for this diagnosis. Dif-
fuse non-nodular infiltration is one; the other is heavy endothelial parasitization by *M. leprae*. Neither finding is specific for Latapi’s lepromatosis, but the presence of dermal nodules or the absence of endothelial parasitization virtually excludes the possibility of Latapi’s lepromatosis.

Three other distinct findings could be regarded as highly suggestive of Latapi’s lepromatosis. These are 1) telangiectasias, either eruptive or as mats on the face and chest, 2) palpable but not visible subcutaneous plaques, and 3) as manifestations of diffuse infiltration, widening of the nasal root, poorly defined induration of the facial cheeks, with or without erythema, and swelling of the backs of the hands.

In the presence of the two “sine qua non”’s other common changes could be regarded as supportive of a diagnosis of Latapi’s lepromatosis. These include complete eyebrow and or eyelash alopecia, nasal septum perforation, and significant S-GPSI with little motor change.

In lepromatous patients who present without nodular change and who have heavy parasitization of endothelial cells, (in our experience those who present with spontaneous ENL (24)), findings which point away from a diagnosis of Latapi’s lepromatosis include normal eyebrows and eyelashes, mild or absent rhinitis, and ocular involvement.

Five biopsy specimens obtained before the onset of Lucio’s phenomenon were examined by one of us, three being available for review. In all four with a Fite stain, endothelial parasitization by *M. leprae* was evident. As demonstrated by hematoxylin and eosin, three histologic patterns were identified, and each pattern could be related to the clinical findings at the biopsy site. In Cases 1 and 2, where the specimen chosen was from clinically normal skin, the infiltrate was scant, and with little if any other inflammatory change, aptly described as “apparently normal.” In Case 4, with the clinical findings of erythematous nodules, a distinct lymphocytic infiltrate was associated with foamy macrophages. In cases 3 and 5, the specimens being obtained from not visible, non-tender, indurated subcutaneous plaques, well developed vascular changes were associated with a heavy infiltrate of macrophages.

Two of the 3 pre-Lucio specimens available for review, from cases 3 and 5, demonstrated endothelial proliferation with lumen occlusion, and were obtained 7 and 5 months, respectively, before the onset on Lucio’s phenomenon. In the third specimen, obtained 4 years before the onset of the Lucio’s phenomenon, no such vascular change was evident.

The most conspicuous disagreement in the literature concerning Lucio’s phenomenon is in regard to its histologic pattern, leukocytoclastic vasculitis (LCV): LCV yes (2, 18, 31) or LCV no (10, 22, 25)? The review of our biopsy material is in accord with our previous conclusion that the histologic pattern is not that of LCV (25). The most likely explanation for the disagreement is differing criteria for what constitutes LCV. Another possibility is the intellectual difficulty in dissociating or uncoupling the idea of a putatively immune complex disorder of skin (23) from the histologic pattern of LCV. The histologic pattern called LCV is that found in “palpable purpura” and is the same whatever disease may be producing the lesions of “palpable purpura.” The lesions of “palpable purpura” were not found in any of these 30 patients.

Clinically, a variety of septic infarcts and thrombotic syndromes (4) may mimic the infarct of Lucio’s phenomenon, being hemorrhagic and having serrated borders. In addition, one recent case report gives strong evidence that other vasculitic conditions may closely mimic Lucio’s phenomenon. Tang and Yosipovitch (32), in their report of an acute Churg-Strauss syndrome, have in their clinical photograph (their fig. 1) illustrated changes perfectly consistent with the serrated, hemorrhagic infarcts of the Lucio reaction. In the same report is a photomicrograph (their fig. 2) which shows extravasation of erythrocytes, and in our interpretation, congestion of superficial vessels, and a necrotic epidermis, identified in the legend as “scale crust,” a pattern looking much like our Fig. 1d. Viewed in this perspective, the second episode of infarctions in Case 9, would be best regarded as being a Lucio’s phenomenon-like tissue response of unknown cause, not a true Lucio’s phenomenon.

Comfort may be taken from this series, where by the time of development of
Lucio’s phenomenon, overt clinical signs of lepromatous leprosy, without exception, were present. Conversely, anxiety may arise from this series, where, in some cases, the diagnosis of leprosy was not made until the Lucio phenomenon occurred, even though preceding characteristic signs and symptoms, although seen and heard by physicians, were not interpreted as suggesting the possibility of leprosy.

Acknowledgment. Many physicians have provided help in making this report possible. For access to clinical data and histologic material on some of the patients, the authors are indebted to Drs. Lewis Bowman, Randy Burke, and Keith Carlson. Dr. Nancy Warner provided valuable advice and printed the photomicrographs. Dr. John T. Crissey provided valuable advice and printed the photographs in “black and white.” Dr. Claudia Renn translated reference number 1.

REFERENCES


Erythema Nodosum Leprosum and HIV Infection:
A Therapeutic Experience¹

Nand Lal Sharma, Vikram K. Mahajan, Vikas C. Sharma, Sandip Sarin, and Ramesh Chander Sharma²

ABSTRACT

The relationship between leprosy and HIV infection is not yet fully understood, as not much is known about the natural history of the co-infected patients. The matter has become more confusing because of conflicting reports. Type-1 lepra reactions and neuritis appear to be severe and more frequent among them. But erythema nodosum leprosum too is not as uncommon among these patients as it was once thought.

Management of these co-infected patients is often difficult for want of clear-cut guidelines on clinical care. We report here our experience of treating recurrent, severe erythema nodosum leprosum in a patient concurrently having leprosy and HIV infection. Early institution of antiretroviral therapy appears to provide an edge in improving the therapeutic outcome for him. It also suggests a direct and more complex interplay of HIV and Mycobacterium leprae infection.

RÉSUMÉ

La relation entre la lèpre et l’infection par le VIH n’est pas encore complètement comprise, comme peu de choses sont connues sur l’histoire des patients co-infectés. Le sujet est devenu d’autant plus confus que des rapports contradictoires ont été publiés. Les réactions lépreuses de type 1 et les névrites seraient plus sévère et fréquentes parmi les patients co-infectés. Mais l’érythème noueux lépreux n’est pas aussi rare parmi ces patients que ce qui avait été originellement considéré.

La prise en charge clinique de ces patients co-infectés est souvent difficile, car elle manque de recommandations claires pour le traitement. Nous rapportons ici notre expérience à traiter un érythème noueux lépreux récurrent et sévère chez un patient souffrant concomitamment de lèpre et d’infection par le VIH. La mise en ?uvre rapide d’une thérapie anti-rétrovirus semble avoir joué un rôle pivot dans l’amélioration du résultat thérapeutique chez ce patient. Cela suggère une interaction directe et plus complexe entre les infections par Mycobacterium leprae et le VIH.

RESUMEN

La relación entre la lepra y la infección por el VIH no está completamente entendida, como tampoco se conoce mucho acerca de la historia natural de los pacientes co-infectados. El tema ha llegado a ser todavía más confuso debido a la publicación de reportes contradictorios. Las reacciones tipo-1 de la lepra y la neuritis parecen ser graves y muy frecuentes entre los pacientes co-infectados pero el eritema nodoso leproso no es tan raro como antes se creía.

El manejo de estos pacientes co-infectados a menudo es difícil debido a la ausencia de lineamientos claros sobre su tratamiento y cuidado clínico. Aquí, nosotros reportamos nuestra experiencia sobre el tratamiento recurrente de eritema nodoso leproso grave en un paciente con lepra co-infectado por el VIH. La administración temprana de la terapia anti-retroviral favoreció el resultado de la terapia general en el paciente. Se analiza el caso y se reconoce el interjuego directo y muy complejo entre el VIH y la infección por M. leprae.

¹Received for publication on Dec. 13, 2004. Accepted for publication on May 20, 2005.
Reprint requests to: Dr. N. L. Sharma, Department of Dermatology, Venereology and Leprosy, Indira Gandhi Medical College, Shimla 171001 (H.P.), India; e-mail: nandlals@hotmail.com
The relationship between leprosy and HIV infection remains obscure due to conflicting reports appearing over a period of time. By analogy with the development of active tuberculosis and other mycobacterial infections among HIV positive patients, an increased prevalence of leprosy was expected, particularly towards lepromatous spectrum, and possibly also the prevalence of erythema nodosum leprosum (ENL) reaction, in areas where both leprosy and HIV are endemic. Earlier literature is also replete with reports on increased frequency of Type-1 lepra (reversal) reactions, severe neuritis, poor therapeutic outcome and recurrences among HIV infected leprosy patients (5, 9, 23), without reports on ENL reaction. More recent data, however, show that HIV infection has no significant effect on epidemiology, clinical, histological, and immunological spectrum of leprosy (4). Reports on ENL among HIV positive leprosy patients, too, have started to appear (13, 14). Studies on granuloma formation and immune patterns in co-infected patients reveal no greater risk for development of multibacillary (MB) leprosy or ENL and, rather contrary to expectations, a satisfactory response to antileprosy treatment has been recorded (13).

Due to lack of information on the natural history of co-infected patients and the absence of guidelines for the management of such cases, it is often a challenge to the ingenuity of the treating clinician. We report here our experience treating recurrent, severe ENL in a leprosy patient concurrently having HIV infection without HIV-related clinical disease.

**Case Report.** A 30-year-old male was hospitalized with recurrent episodes of multiple, erythematous, painful, widely spread cutaneous lesions of 4-month duration. Each episode was accompanied by fever, malaise, body aches, arthralgia, and ankle edema. Some of these lesions had also developed into necrotic crusted ulcers. Antibiotics and anti-inflammatory drugs would temporarily improve his condition. He also reported promiscuous sexual behavior. He was febrile (102°F). Dermatological examination showed diffuse infiltration, numerous, erythematous, tender papulo-nodular lesions involving the whole body except for palms, soles, and scalp. Some of them showed central necrotic sloughing and crusting. Atrophic scars of previously healed lesions were also noted. His conjunctivae were congested. Hair, nails, and oropharynx were normal. He had no significant lymphadenopathy. Asymmetric, tender thickening of all peripheral nerve trunks along with corresponding hypoesthesia was noted over hands and feet. Slit-skin smear examination from 5 sites (World Health Organization, W.H.O.) showed 6+ BI. Ophthalmic, CNS, CVS, pulmonary and abdominal examination revealed no abnormality and there was no historical or clinical evidence of opportunistic infections. The erythrocyte sedimentation rate was 50 mm in first hour and other laboratory studies including complete blood counts, hepato-renal function tests, urinalysis, chest radiograph, VDRL and Treponema pallidum haemagglutination tests showed no abnormality. He was HIV positive by ACON rapid card chromatographic immunoassay (ACON Biotech Hangzhou Co. Ltd., China), Capillus direct latex agglutination assay (Trinity Biotech U.S.A., N.Y. 14702-1059) and Genedia HIV ELISA test (Greencross Life Sciences Corp., Korea). His CD4+ and CD8+ cell counts were 798 and 1541 cells/microl, respectively, using the Fluorescence Activated Cell Sorter counting system (Beckton Dickenson Immunocytometry Systems, California, U.S.A.), and the CD4 : CD8 ratio was 0.52 (Normal Ranges = 865 CD4+, 552 CD8+ cells/microl and CD4 : CD8 ratio 1.7) (15). He did not consent for biopsy and could not afford the cost of viral load studies.

**Clinical progress.** The clinical diagnosis was lepromatous leprosy with recurrent, severe ENL and HIV infection, and the patient was initially given W.H.O. MB multi-drug therapy (M.D.T.) along with prednisolone 60 mg/d, ibuprofen 400 mg t.i.d. and colchicine 0.5 mg b.i.d. Over the next 2 weeks, healing of lesions and symptomatic improvement occurred without recurrences. Subsequently, when the dose of prednisolone was tapered off to 40 mg/d, fresh ENL lesions, ulnar nerve neuritis (without sensory motor deterioration) and systemic symptoms reappeared. He did not improve in spite of increasing the dose of prednisolone to 80 mg/d. At this juncture colchicine was stopped and thalidomide 100
mg b.i.d. was added with the plan to taper off prednisolone once the remission was achieved. The ENL and nerve tenderness decreased within a week and tapering of prednisolone was started. All the while, the patient continued to receive MB-M.D.T., thalidomide 100 mg b.i.d. and other supportive treatment.

After 3 weeks of thalidomide therapy, while still on prednisolone (30 mg/d), he developed dryness of mucosae and a generalized, pruritic, erythematous, diffuse (discrete at places) maculo-papular rash. Initial withdrawal of MB-M.D.T. did not improve the rash. The rash, however, subsided after stopping thalidomide. The dose of prednisolone was increased to 60 mg/day immediately upon recurrence of ENL. The case was reviewed and in view of apparent cushingoid features, as well as poor control of ENL, it was decided to add anti-retroviral treatment (ART) comprising stavudine 30 mg, lamivudine 150 mg and nevirapine 200 mg, all in twice daily doses (the only available and affordable regimen), to the already existing regimen of MB-MDT and oral prednisolone.

His general condition improved, recurrences of ENL stopped and dose of prednisolone could be reduced to 30 mg/d in next 10 days when the patient left the hospital on his own. On a subsequent visit after a month, he was free of ENL, continuing MB-M.D.T. and ART but had stopped prednisolone. However, he was lost to further follow-up.

**DISCUSSION**

As yet there is conflicting and inadequate information on the interactions of HIV and leprosy co-infection. HIV infection was thought to decrease the risk of ENL until recently when reports of ENL among co-infected patients started to appear albeit infrequently. This is contrary to increased frequency of Type-1 lepra reactions and neuritis in co-infected individuals. Nery, et al. (13) observed no enhanced risk of ENL among their patients. In contrast, Gebre, et al. (6) recorded a definite higher risk (relative risk: 5.2; 95% CI 1.7–15.9) of ENL reactions in a prospective study comprising 22 HIV positive leprosy patients. Our patient had severe, recurrent ENL reaction with necrotic lesions. Considering that leprosy has a much longer incubation period the HIV infection in this patient appears a subsequent development. Possibly, like other intercurrent infections, it acted as a trigger for ENL reaction. The ENL also appears to be severe and recurrent in this group of patients similar to Type-1 lepra reactions.

Among HIV seropositive patients neuritis always occurs in association with skin manifestations suggesting that nerve dysfunction is due to Type-1 lepra reaction. HIV is neurotropic and may cause necrotizing vasculitis of the nerves (15). Possibly the interaction of neurotropicity of both *Mycobacterium leprae* and HIV may result in neuropathy that is severe and unresponsive to steroid therapy (5). Vreeburg, et al. (24) also noted that though neuritis is equally common in both HIV positive and HIV negative patients, the therapeutic outcome with steroids was poorer in the HIV positive group. Similarly, HIV-induced vasculopathy might aggravate immune complex mediated vasculitis/panniculitis of ENL that responds poorly to steroid therapy as has been observed in our patient.

Thalidomide, 100–400 mg/d, is currently the recommended drug for recurrent, moderate to severe ENL reactions. Its use has been associated with normalizing effects on TNF, IFN, and helper-suppressor T-cell ratio (20), decreases in dermal infiltration of polymorphonuclear leukocytes and T-cells, and down regulation of the expression of ICAM-1 and MHC-1 antigens on epidermal keratinocytes (20). Its exact mechanism of action in ENL, however, remains unclear. Recently it has also been reported to produce an anti-retroviral effect without any negative effect on immunocompetence (21), possibly through inhibition of TNF production and by blocking TNF stimulated HIV replication (18). Due to these properties it appears to be the drug of choice for treating ENL among HIV infected leprosy patients. However, in view of other reports of increased HIV viral counts caused by this drug it should be used with caution for these patients until further studies are available (17). Apart from its well known teratogenic effects other common adverse reactions like peripheral neuropathy, somnolence and constipation limit its routine use. Hypersensitivity skin rash is uncommon and usually...
appears 2 to 10 days after treatment and subsides after its withdrawal. Thalidomide was effective in our patient also. However, he developed a generalized cutaneous rash in spite of simultaneously receiving 30 mg/d prednisolone. Curiously these patients seem to tolerate the drug poorly and this phenomenon has also been previously correlated with lower numbers of pre-existing CD4+ cells (20). The predominance of CD8+ cells in lepromatous lesions as compared to predominance of CD4+ cells in the typical granulomatous response seen in tuberculoid leprosy lesions (13) and the presence of ICAM 1, HLA DR, TNF, IFN at lesional sites suggests no difference in immune response in both HIV positive and negative leprosy patients (19, 20). The tissue cellular immunity (CMI) against *M. leprae* appears well preserved irrespective of low CD4 + cell counts in the peripheral blood of HIV infected patients or the stage of HIV infection (13). The exact pathologic mechanism of ENL among these co-infected patients is, however, not fully understood. There is indirect evidence consistent with increased CD4+ lymphocytes activity in ENL (11,23). The relative lack of ENL (immune complex mediated) reaction as compared to reversal (cell mediated) reactions among HIV positive lepromatous cases and no therapeutic effect of thalidomide in reversal reactions, that acts through suppression of helper T-cells activity (12), suggests some kind of involvement of CD4+ lymphocytes in ENL. Furthermore, the loss of lesional CD4+ cell function may not be complete as is postulated in tuberculoid leprosy lesions in HIV infected patients (19). It is also known that CD4+ lymphocytes become depleted as the HIV disease progresses and cytotoxic CD8+ lymphocytes, including various subsets, increase significantly (6). Findings such as these may eventually help to explain the occurrences of ENL among these patients. Furthermore, in the early stages of HIV infection some degree of immunocompetence is retained and the occurrence of ENL in early HIV infection may not be unusual as has been the case in our patient.

In our patient, despite an early HIV infection and near normal CD4+/CD8+ counts (the low CD4+ : CD8+ ratio is apparently an artifact of high CD8+ counts), the severe ENL reaction showed poor control even with higher doses of corticosteroids. Addition of ART not only improved the therapeutic response to lower doses of steroids but also helped in their complete withdrawal subsequently. We make no attempt to speculate about the mechanisms underlying our observation. However, clinicians must bear in mind the possibility of precipitating Type-1 lepra reactions during ART/HAART due to immune reconstitution (8).

REFERENCES
7. Grossman, Z., MIEBER-SCHELLESHEIM, M., SOUSA, A. E., VICTORINO, R. M., and PAUL, W. E. CD4+ T-cell depletion in HIV infected patients (19). It is also known that CD4+ lymphocytes become depleted as the HIV disease progresses and cytotoxic CD8+ lymphocytes, including various subsets, increase significantly (6). Findings such as these may eventually help to explain the occurrences of ENL among these patients. Furthermore, in the early stages of HIV infection some degree of immunocompetence is retained and the occurrence of ENL in early HIV infection may not be unusual as has been the case in our patient.

In our patient, despite an early HIV infection and near normal CD4+/CD8+ counts (the low CD4+:CD8+ ratio is apparently an artifact of high CD8+ counts), the severe ENL reaction showed poor control even with higher doses of corticosteroids. Addition of ART not only improved the therapeutic response to lower doses of steroids but also helped in their complete withdrawal subsequently. We make no attempt to speculate about the mechanisms underlying our observation. However, clinicians must bear in mind the possibility of precipitating Type-1 lepra reactions during ART/HAART due to immune reconstitution (8).

REFERENCES
7. GROSSMAN, Z., MIEBER-SCHELLESHEIM, M., SOUSA, A. E., VICTORINO, R. M., and PAUL, W. E. CD4+ T-cell depletion in HIV infected patients (19). It is also known that CD4+ lymphocytes become depleted as the HIV disease progresses and cytotoxic CD8+ lymphocytes, including various subsets, increase significantly (6). Findings such as these may eventually help to explain the occurrences of ENL among these patients. Furthermore, in the early stages of HIV infection some degree of immunocompetence is retained and the occurrence of ENL in early HIV infection may not be unusual as has been the case in our patient.


Effects of Purification and Fluorescent Staining on Viability of *Mycobacterium leprae*

Ramanuj Lahiri, Baljit Randhawa, and James L. Krahenbuhl

**ABSTRACT**

Over the years, researchers have carried out experiments with *Mycobacterium leprae* obtained from either human multibacillary lesions, or infected armadillo tissues, or infected footpad tissues of conventional mice as well as athymic nu/nu mice. In general, these sources of leprosy bacilli are satisfactory for most biochemical and mouse footpad studies, but less than satisfactory for studies in cell biology and immunology where contaminating host tissues pose a serious problem. We examined the utility of a procedure for eliminating mouse footpad tissue from *M. leprae* suspension using sodium hydroxide solution and its subsequent effect on the viability of the organism by determining the rate of palmitic acid oxidation, bacterial membrane integrity, and growth in the mouse footpad. We found that treating *M. leprae* suspension, obtained from infected nu/nu mouse footpad, with 0.1N NaOH for 3 min was sufficient to remove the majority of mouse tissue without adversely affecting the viability of the organism. This is a simple and rapid method to get suspensions of nu/nu footpad-derived viable *M. leprae* essentially free of host tissues, which can be a research reagent for studying the host-pathogen relationship in leprosy. We also report here a method for labeling *M. leprae* with the fluorescent dye PKH26, without compromising on the viability of the organism. This method may be useful in intracellular trafficking studies of *M. leprae* or in other cell biology studies that require tracking of the bacteria using fluorescent tag. We observed the staining to be stable *in vitro* over considerable lengths of time and did not affect the viability of the bacteria.

**RÉSUMÉ**

Depuis des années, les chercheurs ont mené des expériences à partir de *Mycobacterium leprae* issues de lésions multibacillaires humaines, de tissus infectés de tatous à neuf bandes ou bien de tissu de coussinets plantaires de souris tant conventionnelles que nues athymiques (nu/nu). Ces sources de bacilles de Hansen sont en général satisfaisantes pour la plupart des études biochimiques et d’inoculation au coussinet plantaire de souris, mais pas satisfaisantes pour les études de biologie cellulaire et d’immunologie, où les éléments contaminants provenant de l’hôte peuvent représenter un sérieux problème. Nous avons vérifié l’utilité d’une procédure à base de soude pour éliminer les tissus plantaires de souris contaminant les suspensions de *M. leprae*, en vérifiant son effet sur la viabilité de la bactérie par la détermination du taux d’oxydation de l’acide palmitique, de l’intégrité de la membrane de la bactérie et de la croissance dans le coussinet plantaire de souris. Nous avons trouvé que le traitement pendant 3 minutes avec 0,1 N NaOH, de suspensions de *M. leprae* obtenues à partir de coussinets plantaires de souris nu/nu, était suffisant pour enlever la majorité des tissus de souris sans pour autant affecter de façon adverse la viabilité de l’organisme. C’est une méthode simple et rapide, qui permet d’obtenir des suspensions viables de *M. leprae* à partir de lépromes de souris nu/nu dévolus presque entièrement de tissus de l’hôte, représentant de meilleurs réactifs de recherche pour étudier la relation hôte-pathogène de la lèpre. Nous rapportons également ici une méthode pour marquer les *M. leprae* avec le produit fluorescent PKH26, sans compromettre la viabilité du microorganisme. Cette méthode peut être utile pour étudier les mouvements et localisations intracellulaires de *M. leprae* ou bien des études de biologie cellulaire, où le marquage de la bactérie par un colorant fluorescent est requis, afin de pouvoir la suivre. Nous avons constaté que le marquage était stable *in vitro* pendant des temps importants et n’affectait pas la viabilité de la bactérie.

---

1 Received for publication on Feb. 28, 2005. Accepted for publication on May 15, 2005.
2 R. Lahiri, Ph.D.; B. Randhawa, B.S.; and J. L. Krahenbuhl, Ph.D. Laboratory Research Branch, National Hansen’s Disease Programs, Louisiana State University, Baton Rouge, 70803 U.S.A.
Reprint requests to: James L. Krahenbuhl, Ph.D., Laboratory Research Branch, National Hansen’s Disease Programs, Louisiana State University, Skip Bertman Drive, Baton Rouge, LA 70803, U.S.A. E-mail: jkrahe1@lsu.edu
Over one hundred thirty years after its discovery as the causative agent of leprosy, *Mycobacterium leprae* is yet to be cultured *in vitro*. This obstacle has not stymied experimentation with leprosy bacilli of human origin or from infected armadillos or the mouse footpad, although adequate numbers, purity, and questionable viability of *M. leprae* have affected experimental reproducibility and presented additional obstacles to researchers.

In order to have weekly access to large numbers of highly viable *M. leprae*, we maintain several isolates in serial passages in athymic nu/nu mice where growth in the footpad routinely produces a few billion organisms. We have adapted radiorespirometry (RR) procedures to measure oxidation of radiolabeled palmitic acid \(^{15}\) to compare viability of different suspensions of *M. leprae* as defined by metabolic activity. RR was shown to correlate well with growth in the mouse footpad (MFP) \(^{22}\). Recently, we have employed evaluation of the membrane integrity of individual *M. leprae* in a suspension with LIVE/DEAD BacLight Bacterial Viability Staining (VS) Kit\(^{\circ}\) as an additional assay of bacterial viability on the assumption that the bacilli with damaged membrane are dead \(^{11}\). However, even nu/nu MFP derived *M. leprae* suffer from one drawback and that is the presence of contaminating mouse tissue in the bacterial suspension. While, this contaminating host tissue does not affect subsequent passage to a new host or some *in vitro* investigations, it is absolutely not desirable in studies designed to observe immunological reactions, intracellular trafficking, and pathogenesis of the disease, which is markedly different from that of tuberculosis. Slow speed centrifugation removes larger pieces of mouse tissue from the suspension, but counter-staining of the acid-fast bacilli (AFB) gives clear evidence for unacceptable levels of remaining mouse tissue in the suspension.

In this study, we examined the utility of a procedure for eliminating mouse footpad tissue from *M. leprae* suspension using sodium hydroxide (NaOH) solution and its subsequent effect on the viability of the organism as defined by RR and VS. We also report here a method for labeling *M. leprae* with the colorant fluorescent PKH26 that can be used to study the viability of the organism *in vitro* and in other studies of the biology of *M. leprae*.

**METHODS AND MATERIALS**

*Nude mouse-derived M. leprae*. *Mycobacterium leprae* (isolate Thai-53) is maintained in serial passage in the footpads of athymic nu/nu mice (Harlan, Indianapolis, Indiana, U.S.A.). Mice were inoculated on the plantar surface of both hind feet with 5
× 10^7 fresh, viable nu/nu-derived \textit{M. leprae}. When the mouse footpads became moderately enlarged (at ~6 months), they were harvested for intracellular \textit{M. leprae} as described previously (22), washed by centrifugation (18,000 g for 30 min), resuspended in either medium 7H12 or RPMI-1640 (Gibco Invitrogen, Carlsbad, California, U.S.A.) + 10% (v/v) fetal calf serum [(FCS) Gibco Invitrogen, Carlsbad, California, U.S.A.], enumerated by direct count according to Shepard’s method (18) and held overnight at 4°C, pending quality control testing for contamination. The bacterial suspension was passed 3 to 4 times through a 27G needle prior to counting in order to remove clumps. Freshly harvested bacilli were always employed in experiments (within 24 hr of harvest).

**NaOH treatment of \textit{M. leprae}**. 1 × 10^9 fresh \textit{M. leprae} were resuspended in 1.0 ml of the appropriate concentration of NaOH [0.1N–0.9N] (Sigma, St. Louis, Missouri, U.S.A.) and incubated for 3 min at room temperature, after which the bacteria were washed (10,000 g for 5 min at 4°C) thrice in 7H12 medium and finally resuspended in appropriate media. There was a 30 to 50% loss of bacteria in this process.

**Scanning Electron Microscopy**. Ten μl suspension (1 × 10^9/ml) of 0.1N NaOH treated or untreated \textit{M. leprae} were spread on poly-lysine coated plastic cover slips, air dried, fixed, and washed prior to 1% Osmium tetraoxide treatment. Following which the cover slips were washed in deionized water and then dehydrated by several changes of 30% to 100% ethyl alcohol. After dehydration the samples were subject to critical point drying prior to sputter coating with gold and palladium. The samples were then visualized in a FEI Quanta 200 scanning electron microscope.

**Radiorespirometry**. 1 × 10^7 \textit{M. leprae} were inoculated into 1.0 ml of BACTEC 7H12B media (Becton Dickinson, Franklin Lakes, New Jersey, U.S.A.) containing 14C-palmitic acid in a loosely capped vial which, in turn, was inserted into a wide mouth liquid scintillation vial lined with filter paper impregnated with NaOH, 2,5-diphenyloxazole (Sigma, St. Louis, Missouri, U.S.A.) and Concentrate I (Kodak, Rochester, New York, U.S.A.) and incubated at 33°C. When read daily, captured 14CO₂ determines the rate of 14C-palmitic acid oxidation (9). In the present study, on the seventh day cumulative counts per minute (CPM) are reported.

**Fluorescent staining for assessing bacterial membrane integrity**. The membrane integrity of individual \textit{M. leprae} in a suspension was evaluated with LIVE/DEAD BacLight Bacterial Viability Staining (VS Kit® (Molecular Probes, Eugene, Oregon, U.S.A.) as described previously (19). Briefly, \textit{M. leprae} were washed (10,000 g for 5 min) in normal saline and incubated for 15 min at room temperature with 6 μM Syto9 and 30 μM propidium iodide (PI). After staining the bacteria were resuspended in 10% (v/v) glycerol in normal saline, passed through 27G needle to dissociate clumps, and the percentage of dead and live bacteria in the suspension were enumerated by direct counting of fluorescent green and red bacilli using appropriate single bandpass filter sets.

At least 200 individual bacteria or 10 microscopic fields, whichever was more, were counted to evaluate the percentage of bacteria having membrane damage in the suspension.

**Staining with PKH dyes**. Freshly harvested \textit{M. leprae} treated with 0.1N NaOH (1 × 10^9) were resuspended in 1.0 ml of the provided “diluent C” and then stained for 2 min at room temperature with a 1:250 dilution of either PKH26 (red) or PKH67 (green) dye (Sigma, St. Louis, Missouri, U.S.A.). After 2 min the staining was halted by adding an equal volume of FCS. The suspension was washed (10,000 g for 5 min) thrice in appropriate medium. The numbers of bacteria were recounted following staining by Shepard’s direct count method (18).

**Macrophage culture**. Resident peritoneal cells from Swiss mice were harvested and allowed to adhere for at least 6 hr at 37°C and 5% (v/v) CO₂, on plastic cover slips in 24 well tissue culture plates (Corning, Corning, New York, U.S.A.) as previously described (14). After washing to remove non-adherent cells, the adherent cells were infected overnight at 33°C with PKH26 stained \textit{M. leprae} at a multiplicity of infection of 20:1. At the end of the incubation extracellular \textit{M. leprae} were removed by washing the cover slips.
Footpad growth of *M. leprae*. BALB/c mice, 5 in each group, were inoculated on the plantar surface of both hind feet with $1 \times 10^4$ PKH26 stained or control *M. leprae*. At 3 and 6 months both hind footpads were harvested, processed and the number of AFB enumerated using Shepard’s technique.

Statistical analysis. The data are shown as means ± standard deviation (S.D.) from a representative of three to four experiments. The raw data were subjected to one-tailed or two-tailed Student’s *t* test to determine whether the observed differences between the means were significant. *p* <0.05 was taken as significant.

RESULTS

**Scanning electron microscopy of NaOH treated *M. leprae*.** To observe the effects of NaOH treatment on the appearance of a suspension of nu/nu footpad derived *M. leprae*, suspensions were treated for 3 min with 0.1N NaOH, washed and observed with the S.E.M. The results (Fig. 1) showed that treatment resulted in a marked clearance of mouse footpad tissues from the *M. leprae* suspension in comparison to untreated controls. We did not observe any significant improvement in the quality of the *M. leprae* suspension, by scanning electron microscopy, following treatment with 0.1N NaOH for a longer period or with higher concentrations of NaOH (data not shown).

**Effects of NaOH treatment on metabolic activity of *M. leprae*.** To determine the effects of NaOH treatment on sustained *in vitro* metabolic activity, *M. leprae* were treated with 0.1N, 0.3N, 0.6N, or 0.9N NaOH for 3 min at room temperature, washed in 7H12 medium and prepared for RR. The seventh day RR data (Fig. 2) showed no significant differences in the cumulative oxidation of radiolabeled palmitic acid between the control and the NaOH treated *M. leprae*. However, a significant fall in the RR was observed when the 0.6N or higher concentration NaOH treatment was carried out for longer than 7 min (data not shown).

**Effects of NaOH treatment on membrane integrity of *M. leprae*.** To assess the effects of different NaOH treatments on the membrane integrity of *M. leprae* BacLight fluorescent staining was done. In this assay all the bacilli in the suspension stain with Syto9 (green), i.e., both those with intact membranes as well as those with damaged membranes but the bacilli having damaged...
membrane also stain with PI (red). This assay assumes that all the bacteria having damaged membrane (staining red) are non-viable or dead. The data (Fig. 3) clearly showed that 3 min treatment with 0.1N, 0.3N or 0.6N NaOH did not impart any significant membrane damage. However, treatment with 0.9N NaOH for the same duration resulted in a significant decrease (p <0.0001) in the number of \( M. leprae \) having intact membrane.

**Staining of \( M. leprae \) with PKH26 dye.**
The staining of \( M. leprae \) with PKH26 dye was done after treating the suspension of bacilli with NaOH. We chose the 3 min treatment with 0.1N NaOH as described above. A 1:250 dilution of PKH26 dye provided bright red fluorescent bacteria that maintained solid fluorescence for at least 15 days when held in vitro in dark at 4°C (data not shown). Similar findings were observed when the green (PKH67) dye was employed. We used 3 different dilutions of the dye (1:250, 1:500 and 1:1000) and followed the manufacturer’s protocol for staining \( M. leprae \) and found no detrimental effects of PKH26 staining on subsequent palmitic acid metabolism as measured by RR (Fig. 4).

**Uptake of PKH26 stained \( M. leprae \) by mouse peritoneal macrophages.** To visualize intracellular fluorescent bacilli, adherent mouse peritoneal macrophages were infected in vitro with either PKH26 stained or unstained live \( M. leprae \) at a MOI of 20:1. We did not observe any difference in the up-
take of PKH26 stained *M. leprae* when compared to that of unstained control (Fig. 5).

**Growth of PKH26 stained *M. leprae* in mouse footpads.** One $\times 10^4$ PKH26 stained or unstained *M. leprae* were used to infect each hind footpad of BALB/c mice. The footpads were harvested at 3 and 6 months and the total number of AFB per footpad were counted. The results (Fig. 6) indicate no significant difference between the growth kinetics of the PKH26 stained *M. leprae* ($3.3 \times 10^6 \pm 1.3 \times 10^6$ AFB/footpad at 6 months) to that of the control ($1.8 \times 10^6 \pm 0.7 \times 10^6$ AFB/footpad at 6 months).

**DISCUSSION**

Over the years, researchers have carried out experiments with *M. leprae* obtained from a variety of sources, including the nodules or lesions from multibacillary (MB) patients, infected armadillo tissue, and infected footpads from conventional mice as well as immunocompromised neonatal thymectomized, lethally irradiated (NTLR) and nu/nu mice. In general, these sources of leprosy bacilli have proved satisfactory for MFP studies but less than satisfactory for *in vitro* experiments.

*M. leprae* from infected human patients are difficult to obtain and there is no control of the investigator over the quality of these bacilli. Human derived bacilli were obtained from untreated cases if viable organisms were required ($^4,^7$), but the quality (viability) of these bacilli was poor and human biopsies were an inconsistent source of organisms as it would be unethical to withhold treatment from an identified case of MB leprosy solely to provide a source of bacilli. The quality of bacilli obtained from passage of *M. leprae* in the conventional MFP model was more consistent than that from human origin. However, though conventional MFP model yielded adequate numbers of bacilli for a variety of additional MFP studies ($^{16}$), these organisms were unsatisfactory for most *in vitro* studies as they were too few (maximum yield of $\sim 1 \times 10^6$ per footpad) and consisted largely of footpad tissue. A single infected armadillo can yield tens of billions of bacteria ($^{10}$), but the quality of bacilli in terms of viability remains poor (unpublished results). Hence, armadillo derived *M. leprae* are good for conducting certain biochemical studies but not for *in vitro* or cell culture studies where the viability of the bacterial inoculum can dictate the outcome of the experiments. For the latter kind of studies athymic nu/nu footpad-derived *M. leprae* is best, as a single mouse can yield a few billion highly viable bacteria.

The present report establishes a simple purification method for removing host tissue from suspensions of *M. leprae* without affecting the viability of the bacilli. Other procedures have been developed to purify tissue derived *M. leprae* on a large scale but the effects of these treatments on viability of the bacilli was never determined. Armadillo infected liver, spleen and lymph node tissue harbors billions of *M. leprae*($^{10}$) and a two phase method devised by Draper for isolation of pure bacilli from large quantities of infected armadillo liver and spleen was developed($^{17}$) and is used routinely for the provision of the enormous numbers of bacilli required to isolate and characterize cell wall and other *M. leprae* constituents ($^8$). However, until very recently the infected tissues were irradiated with $2.5 \times 10^6$ rads($^{10}$), a dose that kills the bacilli($^1$) making the issue of viable, armadillo-derived *M. leprae* moot. In recent years these tissues have not been irradiated but our studies, employing both RR as a measure of metabolic activity and the BACTLIGHT stain for membrane integrity and MFP challenge show that the viability of even un-irradiated armadillo-derived *M. leprae* is extremely low (unpublished results).

Another important consideration for the provision of *M. leprae* as a research reagent is that armadillos are too expensive to
maintain (1 to 2 years) as a source of bacilli for routine (weekly) experimentation. Armadillos are infected experimentally to provide maximum numbers of *M. leprae* upon harvest, a goal we have found to be inconsistent with providing highly viable organisms harvested during their log phase of growth(13). *M. leprae*-infected athymic nu/nu mice on the other hand are readily available, far less expensive than armadillos to maintain and the infected footpads of individual mice can be harvested weekly to yield billions of bacilli in the crude homogenates of the infected footpad tissues.

Our laboratory is committed to characterizing nu/nu-derived *M. leprae* as a research resource and we have described their response to physical-chemical treatment including susceptibility to ionizing(1) and UV(21) radiation, effects of deep-freeze storage and incubation temperatures and response to various fixatives(22, 11).

Previously we employed a Percoll density gradient separation of nu/nu derived *M. leprae* to yield pure suspensions of bacilli that were enriched for viability as defined by RR but losses of total bacilli were routinely >90%, an unacceptable yield. Treatment with 0.1N NaOH has been routinely employed to purify *M. leprae* of host tissue for studying the enzyme activity(13) and isolation of bacterial components(9). The present S.E.M. studies clearly show that brief treatment of nu/nu mouse footpad derived *M. leprae* with 0.1N NaOH eliminates mouse footpad tissue providing a pure suspension of bacilli for potential *in vitro* use as a leprosy research reagent. But a purified reagent is only a partial fulfillment of the needs of researchers; a pure and viable reagent is needed. For example, previous studies from this laboratory have described marked differences in afferent and efferent function of *M. leprae* infected macrophages, depending on whether infection was carried out with viable or non-viable bacilli(19).

The present study investigated the effects of NaOH treatment on subsequent viability and shows that this method of purification does not affect their viability as defined *in vitro* by RR, a measure of metabolic activity that correlates well with growth in mouse footpad(22). To further characterize viable *M. leprae* as a research reagent we have recently adapted the VS procedure to permit evaluation of the viability of individual leprosy bacilli as defined by membrane integrity(11). Interestingly we found that a consistent, though not significant, increase in the cumulative seventh day RR counts and percentage live in VS assay was observed after treatment of bacterial suspension with 0.1N and 0.3N NaOH. These findings may be due to removal of some non-viable bacteria from the suspension by the NaOH treatment. The yield of bacteria after the NaOH treatment and subsequent washings was routinely between 30 and 50%.

Combining RR analysis with VS has allowed us to adjust our routine passage of *M. leprae* in the nu/nu mouse to maximize the viability of a harvested suspension and minimize the duration of the infection(22, 11). In the infected nude mouse the footpad increases in size as bacillary numbers increase markedly and host cells become gorged with *M. leprae*. Viability as measured by RR correlated with MFP growth and was significantly correlated with time in tissue and the number of bacilli per gram of granuloma(22). Very large footpads with high numbers of *M. leprae* per gram of tissue yield less viable bacilli. Highest viability for nude mouse derived *M. leprae* is associated with short to moderate periods *in vivo*. Thus routine short term passage is the best means to assure plentiful, viable stocks of *M. leprae*. The present study extends our interests in defining the properties of viable *M. leprae* as a research reagent.

Access to a reliable source of large numbers of pure, viable *M. leprae* would be an important research resource for today’s leprosy researcher especially those interested in pursuing the cell biology of intracellular infection with *M. leprae* and the unique relationship between the leprosy bacillus and its host cell. A major tool became available to cell biologists a dozen or so years ago with the development of fluorescent tracker dyes which allowed the stable labeling of mammalian cells(20). The technology was based on the incorporation of highly aliphatic reporter molecules containing fluorochrome groups into the lipid bilayers of cytoplasmic membranes. A key feature of these dyes is their retention. Once incorporated, they are trapped in the membrane by
virtue of their inherent insolubility in aqueous solutions. In a variety of eukaryote cells these dyes have been shown to be stable and non-toxic, permitting tracking of adaptively transferred cells in vivo without interfering with their function, for example cytotoxicity\(^{(12)}\). The tracking dyes do not interfere with doubling times of labeled cells and the dye appears to be equally partitioned between daughter cells when a labeled cell divides\(^{(5)}\). Similar dyes have been employed as fluorescent trackers to label prokaryote cells such as protozoa\(^{(15)}\) and bacteria\(^{(3)}\).

In the present studies, the short term effects of staining with PKH26 tracker dye on the metabolic activity of \(M. leprae\) was determined in vitro in axenic media. The growth of stained bacteria in MFP was also determined. Notably, all the PKH dye labeling of bacilli reported in this study was done subsequent to NaOH treatment to remove the contaminating mouse tissues which would also label with the PKH dye. We have also observed that \(M. leprae\) did not label uniformly with PKH dye if mouse tissue was present in the suspension. Therefore, these studies also demonstrated that neither NaOH treatment nor PKH26 labeling of \(M. leprae\) affected the viability of \(M. leprae\) as defined by RR, VS and growth in the mouse footpad, the “gold standard” measurement of the ability of the leprosy bacillus to survive and multiply in vivo. The PKH labeled bacteria can be visualized inside mouse peritoneal macrophages using either fluorescence or confocal microscope. It should be noted, that in cultures where the PKH26 stained \(M. leprae\) were maintained in peritoneal macrophages for more than 7 days there was slight diffusion of the PKH26 dye into the macrophage cytosolic compartments (data not shown).

The NaOH treatment reported here is an easy and fast method to obtain suspensions of nu/nu MFP derived viable \(M. leprae\) essentially free of host tissue, a valuable research reagent required for studying the host pathogen relationship in leprosy. The other research reagent described here is the fluorescently labeled viable \(M. leprae\) which can be utilized in intracellular trafficking studies of \(M. leprae\)\(^{(2)}\) or in other cell biology studies that require tracking of the bacteria using a fluorescent tag. We observed the staining to be stable in vitro over a considerable length of time and did not affect the viability of the bacteria. MFP studies that will explore in more detail the PKH-staining characteristics of multiplying \(M. leprae\) are underway.

Acknowledgement. The authors gratefully acknowledge the expertise of Mr. Greg McCormick in the preparation of S.E.M. photos of \(M. leprae\). These studies were supported by a grant from The American Leprosy Missions, Greenville, SC.

REFERENCES


CASE REPORTS

Borderline Tuberculoid Leprosy with Type 1 Reaction in an HIV Patient—A Phenomenon of Immune Reconstitution

Tarun Narang, Sunil Dogra, and Inderjeet Kaur

The course of leprosy in patients with HIV infection has been a controversial issue for a long time. It is still a matter of debate whether the HIV status of an individual has any impact on the natural history of leprosy and response to anti-leprosy treatment. Though various effects on immune system can be expected in a case of co-existent leprosy with HIV infection, epidemiological studies failed to establish any such bearing in the clinical course of leprosy. A World Health Organization (W.H.O.) meeting in 1993 concluded that there is no convincing evidence for an association between HIV and leprosy (14). A case control study held in South India among leprosy patients also confirmed these findings (11). Individuals with profound immunosuppression due to HIV infection may have active coinfections that are subclinical because of the lack of host inflammatory responses. Reconstitution of the immune system during the initial months of antiretroviral treatment, however, may result in the development of overt clinical manifestations of these coinfections (1,3), as restoration of CD4+ T lymphocytes permits inflammatory responses to be mounted (8,9). Immune reconstitution or immune restoration phenomenon is now a well-recognized complication of highly active antiretroviral treatment (HAART) and has been described in individuals infected with Mycobacterium tuberculosis, nontuberculous mycobacteria, cytomegalovirus, and hepatitis B and C viruses (1,3).

CASE REPORT

A 28-year-old married man was referred to our department for evaluation of erythematous plaque on his left thigh, observed 5 weeks back. He had become aware of his HIV status 4 months back when he was being evaluated for prolonged fever and intractable diarrhoea. His wife was also seropositive. At the time of presentation the patient had advanced immunosuppression, with a blood CD4+ lymphocyte count of 125/µL and a plasma virus load of 150,000 HIV-1 RNA copies/mL. He was started on HAART (zidovudine, lamivudine, and efavirenz) and after 2 months of triple drug therapy, the patient noticed an erythematous plaque on his left thigh. Over the next week, the lesion enlarged, became swollen and smaller lesions appeared in its periphery (The Figure) along with symptoms of pain and paraesthesias in the left leg. Clinical examination revealed well-demarcated, erythematous, edematous tender plaque with complete loss of sensation. Left lateral popliteal nerve was thickened and tender. A clinical diagnosis of borderline tuberculoid (BT) leprosy with reversal (type 1) reaction was made. Skin biopsy confirmed the diagnosis, showing noncaseating granulomas, marked nerve destruction, and no acid-fast bacilli (AFB). At the time that the cutaneous lesions developed, the patient’s plasma viral load had declined to 1750 HIV-1 RNA copies/mL.

Received for publication Feb. 25, 2005. Accepted for publication on June 2, 2005.

1 T. Narang, M.D.; S. Dogra, M.D.; D.N.B., M.N.A.M.S.; and I. Kaur, M.D., M.N.A.M.S., Department of Dermatology, Venereology and Leprology, Postgraduate Institute of Medical Education and Research, Chandigarh, India.

2 Reprint requests to: Dr. Inderjeet Kaur, Additional Professor, Dept. of Dermatology, Venereology and Leprology, PGIMER, Chandigarh-160 012, India; E-mail: kaur_inderjeet@yahoo.com
copies/mL, and his CD4+ lymphocyte count had increased to 280/µL.

The patient was started on W.H.O. multi-drug therapy (M.D.T.) PBR. He was also started on prednisolone 40 mg once daily for the reversal reaction, which was gradually tapered and stopped in 12 weeks. He responded favorably to the treatment.

**DISCUSSION**

HIV has generally not been found to have a significant impact on the clinical course of treated and untreated leprosy. However, it has been reported that the neuritis in co-infected people can be more severe and the reversal reaction may be more frequent after therapy. In endemic areas with HIV disease and leprosy, there does not appear to be a greater incidence of leprosy among HIV patients. It may be because of the very slow proliferation of the bacilli or the prolonged incubation period, or perhaps a particular cellular mechanism involved in its pathogenesis.

HIV-infected patients responding to HAART can show a diverse spectrum of symptoms caused by immune reconstitution and subsequent inflammatory reactions. The pathogenesis of this phenomenon, called immune restoration disease (IRD)/Immune reconstitution inflammatory syndrome (IRIS) is unclear. IRIS is an unusual inflammatory reaction to an opportunistic infection that occurs in a HIV-positive patient with profound immunosuppression during the reconstitution of the immune system in the initial months of HAART (2). A variety of manifestations of IRIS have been described, most prominently including *Mycobacterium avium* complex lymphadenitis, paradoxical exacerbations of pulmonary and CNS *Mycobacterium tuberculosis* infection (9), paradoxical exacerbations of *Cryptococcus neoformans* meningitis, herpes zoster and cytomegalovirus uveitis (12). Reactions in leprosy, especially the type 1/reversal reaction, should be recognized as an IRIS-associated manifestation with a possibility of atypical presentation.

Our patient may well have harbored latent infection with *Mycobacterium leprae* for many years or had ill defined lesions which escaped his attention. He developed clinically apparent leprosy within a span of two months, and the timing of the presentation was related to his immune status. The temporal association between the development of skin lesions and the HAART-induced changes in plasma HIV-1 load and CD4+ lymphocyte count strongly suggests that leprosy manifested clinically as a result of immune reconstitution. Immune reconstitution either resulted in the development of active leprosy or triggered the reversal reaction leading to presentation of previously unrecognized disease. Indeed, many patients with BT leprosy present only when a reversal reaction develops. The onset of immune reconstitution phenomena often occurs within 1 to 6 months of HAART, even prior to substantial increase in the blood CD4+ T lymphocyte count (1,3). Studies have shown that HIV-1 infection is not a risk factor for leprosy (4,6). Although a shift in the spectrum of leprosy from the tuberculoid to the lepromatous form might be expected, studies have shown that HIV-1 co-infection does not alter either the clinical or histological spectrum of the disease (6,9). The development of bordeline tuberculoid (BT) leprosy in this patient (indicating strong cell-mediated immunity), after increase in CD4+ T lymphocyte counts is, therefore, consistent with previous observations (2,5). However, the presentation of leprosy as an immune reconstitution phenomenon does suggest that HIV-1 associated immunosuppression masked the patient’s disease before the start of HAART. There are few case reports of this phenomenon in leprosy (2,5), and it is possible that the incidence of clinically overt leprosy may be decreased among HIV-infected individuals who are profoundly immunosuppressed.

Type 1 (reversal) reactions occur most
frequently among patients with BT leprosy, causing acute inflammation in cutaneous lesions and nerves harboring \textit{M. leprae} antigens. Such reactions result from an increase in cell-mediated immunity and typically occur during the early stages of leprosy treatment. Paradoxically, such reactions are observed more frequently among those with HIV-1 coinfection (13).

The increasing availability of HAART in areas where both HIV and leprosy are prevalent may well reveal latent leprosy cases as a result of an IRIS in patients starting antiretroviral treatment. Differentiation of IRIS from an opportunistic infection is important because IRIS indicates a successful, albeit undesirable, effect of HAART. It is also important to differentiate it from drug toxicity to avoid unnecessary cessation of HAART.

REFERENCES
**Pityriasis versicolor** is a common superficial fungal infection considered in clinical differential diagnosis of leprosy. Studies have reported a higher incidence of *Pityriasis versicolor* in leprosy patients when compared to the general population (4), but there are no reports of co-localization of these two infections. We describe a 24-year-old man with *P. versicolor* lesions localized to the plaque of borderline tuberculoid (BT) leprosy.

A 24-year-old male patient from Bihar, India was diagnosed with BT Hansen and started on multi-drug therapy (M.D.T.) (MBR), rifampicin 600mg and clofazimine 300mg once monthly supervised and clofazimine 50mg with dapsone 100mg daily. Two months after starting M.D.T., he developed type 1 reaction and was started on prednisolone 40 mg once daily, which was tapered after the reaction subsided. He also developed xerosis and ichthyosis after starting M.D.T. (clofazimine induced) and was using coconut oil liberally on the lesions.

Four weeks after the initiation of steroids he presented with hypopigmented itchy lesions appearing over the pre-existing leprosy lesions on the chest. On examination, he had multiple oval to round hypopigmented scaly macular lesions on the large patches of BT disease on his chest and upper back. The lesions were also present in the vicinity of the patch (Fig. 1). However, other lesions of leprosy on the face, arms and lower back did not show any such change. The diagnosis of pityriasis versicolor was confirmed by potassium hydroxide (KOH) examination showing numerous short thick hyphae with clusters of spores. He was given fluconazole 400 mg single dose following which the versicolor lesions cleared in a month (Fig. 2).

*P. versicolor* distribution as normal flora is related to sebaceous gland density, and thus the scalp, face, central chest, and back bear the highest number of fungi (1, 3). High sebum levels, excessive sweating, warm climate, application of oil, malnutrition, administration of systemic steroids, immunosuppressants, and antibiotics are some of the factors that facilitate rapid growth of fungus (2).

Leprosy is characterized by partial or complete destruction of skin appendages including sebaceous glands, so that co-localization of lesions of leprosy and *P. versicolor* is a clinical paradox. Ideally, this
In our case, administration of systemic steroids could be the major factor that promoted the development of *P. versicolor*. However, preferential localization of versicolor lesions to patches of leprosy is perplexing.

**REFERENCES**

In a recent issue of *Nature Medicine*, Krutzik, *et al.* report a novel finding on the role of macrophages and dendritic cells in leprosy. In lepromatous leprosy patients’ blood and lesions, triggering of dendritic cells was impaired, suggesting a defect in the initiation of adaptive immunity.

**INTRODUCTION**

Dendritic cells (DC) and macrophages (Mf) play major roles in innate immunity and provide a first line of defense against invading pathogens like *Mycobacterium leprae*. Mf and particularly DC also play a key role in the onset of subsequent adaptive immunity by triggering pathogen specific T-cell and B-cell responses, and by the formation of immunological memory such that the immune system will be able to remember previous encounters with pathogens later in life. Besides representing key cells of the immune system in combatting bacterial infections, however, Mf and DC, paradoxically also provide a necessary safe-haven for so-called intracellular bacteria, of which mycobacteria in general and *M. leprae* in particular are prime examples: *M. leprae* is widely believed to be unable to dwell outside phagocytes, and exploits these otherwise hostile immune cells to survive and replicate in the human body. Thus, changes in the volatile equilibrium between host and pathogen will dictate whether the host (“immunity”) or the pathogen (“immune escape”) is favored.

Infection of Mf and DC by bacteria is mediated via a series of cell-surface receptors, including Toll-like Receptors (TLR), the Mannose Receptor and the Dendritic cell (DC)-specific surface receptor DC-SIGN (DC-specific ICAM-3–grabbing nonintegrin). These receptors interact with specific biochemical structures on the surface of bacteria and trigger phagocytosis, anti-microbial activity and release of cytokines by the infected cell1–3. DC-SIGN ordinarily interacts with cell surface molecules called ICAM-2 and ICAM-3 that are expressed on a variety of host cells. Interactions between DC-SIGN and ICAM-2 on endothelial cells induce tethering and rolling of immature DCs and thus promotes extravasation of these cells from the blood to inflammatory foci.

Mycobacterial products can trigger TLR-family members and induce immature DCs to differentiate into mature DCs, cells that are specialised in superior induction of T cell mediated immunity. Mature DCs release inflammatory cytokines, highly efficiently present captured antigens to naïve T cells and drive their differentiation and activation into effector and memory T cells. Presentation of antigens to T cells is achieved through surface molecules called MHC class-I and class-II molecules, as well as MHC class-I-like CD1 molecules. MHC class-I and class-II molecules present short protein fragments of pathogens, whereas CD1 molecules present complementary components such as bacterial lipids.

DC are present in very low numbers in the blood (<1%), and have therefore been difficult to study. Most studies on DC have therefore used human blood monocytes that were differentiated *in vitro* into DC-like cells using the cytokines IL-4 and GM-CSF. This technique allows acquisition of much higher cell numbers that are easier to work with. These cells have a DC-like phenotype, express both DC-SIGN and CD1b (DC-SIGN+CD1b−), and are able to activate T cells potently.

**Summary of the Krutzik, *et al.* study**

To their surprise, however, Krutzik, *et al.* now report that this cell type is not observed *in vivo* in the lymphoid tissues they analyzed. Importantly, they find that stimulation of cells via their TLR—as a mimic of bacterial infection-induced differentiation of blood monocytes mostly into either DC-SIGN+CD1b− or DC-SIGN−CD1b+ cells,
whereas only small percentages of double positive cells were seen, the predominant cell type induced by the widely used IL-4/GM-CSF combination. Thus, DC-SIGN and CD1b molecules were expressed mainly on different cell types.

Elegant in vitro studies further revealed that the DC-SIGN*CD1b− cells carried typical Mf markers. The TLR induced expression of DC-SIGN was particularly prominent following exposure to the mycobacterial 19-kDa lipopeptide that binds to TLR2/1, and was mediated by the innate cytokine IL-15. These Mf were able to bind and phagocytose mycobacteria via DC-SIGN, and secreted high levels of inflammatory cytokines which are necessary to activate innate and adaptive immunity. In contrast, TLR induction of DC-SIGN CD1b+ cells was dependent on GM-CSF. These cells resembled DCs and were substantially more capable of activating T cells than DC-SIGN*CD1b− cells. DC-SIGN CD1b+ cells lacked mature DC markers such as CD83, suggesting they had not fully matured yet. Finally, the latter cells were less able to bind BCG compared to the DC-SIGN*CD1b− Mf like cells.

The findings were further extended by examining the expression of these new cell types in leprosy. Much like healthy donors, tuberculoid leprosy patients’ monocytes yielded both DC-SIGN*CD1b− Mf-like and DC-SIGN CD1b+ DC-like cells following TLR activation. A striking finding was that lepromatous patients only yielded Mf but not DC like cells. Such a defect, however, was not observed when the above mentioned IL-4/GM-CSF combination was used to generate monocyte derived “classical” DCs in vitro, ruling out a general defect in their capacity to generate DC-SIGN*CD1b+ DC-like cells at all. Also, normal levels of DC-SIGN CD1b+ DC-like cells were seen in lepromatous patients undergoing reversal reactions. More interestingly, the same cell type distribution was seen in tuberculoid, lepromatous, and reactional lesions. Also in situ, tuberculoid lesions contained both DC-SIGN CD1b+ DC-like cells and DC-SIGN*CD1b− Mf-like cells, whereas lepromatous lesions lacked the latter and mostly contained the former subset. In addition, the Mf cells could be demonstrated to contain *M. leprae* material in lepromatous but not tuberculoid lesions. Thus, since lepromatous patients lack (local) DCs the implication of these findings may be that they are unable to induce and activate proper T cell responses to eradicate *M. leprae*.

**Questions and discussion**

The surprising findings by Krutzik, *et al.* obviously need confirmation and extension in other systems, but are certainly new and provocative. The finding that DC-SIGN+ cells belong mostly to the Mf- but not DC-class is even highly provocative. Nevertheless, some caution may be warranted in overinterpreting this data to indicate that many DC-studies in the past have been performed on cells that hardly or not at all exist in vivo. Before such conclusions can be drawn more work is clearly needed. A small subset of cells in the Krutzik, *et al.* study actually is double-positive (DC-SIGN*CD1b*), both in vitro and in vivo, but may simply be a minor population that could be selectively expanded by IL-4/GM-CSF. It should be pointed out also that various DC and Mf subsets exist (5,6), and that there may even be a continuum of phagocytic cell types, each with its own level of plasticity. This would even further allow these cells to adapt to various conditions and acquire different phenotypes depending on the precise (cytokine-) environment (5). The micro environment in leprosy skin lesions or tonsils may not be ideal to favor “double positive DCs,” but this does not exclude their existence or relevance in the human immune system. Of interest, also other Mf like subsets (CD16*DC-SIGN*; unfortunately, it is not indicated whether these cells were CD1b+*) were found in lepromatous lesions, pointing to the existence of a more complex local repertoire of phagocytic cells in leprosy lesions.

The sample size of patients and lesions studied by Krutzik, *et al.* seems rather small (the numbers of samples studied are not always clearly indicated in the manuscript) so that it is as yet uncertain to what extent the findings in the individuals analysed can be generalised to human leprosy *per se*.

The mechanisms behind the impairment of lepromatous patients’ cells in inducing DC like cells remains unexplained. It is important to resolve this, as this may provide
novel therapeutic angles. The question is what phenotype would result when cells would have been stimulated more physiologically with M. leprae instead of unrelated TLR stimuli, but no data are reported on this issue. Furthermore, a general impairment in DC function in lepromatous leprosy is not easily reconciled with the characteristic and rather specific defect in T-cell responsiveness to antigens of M. leprae in lepromatous leprosy: general DC defects would be expected to lead to more general defect in T-cell responses, but this is not typically the case in lepromatous leprosy.

It also remains unknown if the defect in local DC like cells in lepromatous leprosy lesions is permanent or not: is this defect disease-activity dependent, or is it rather a permanent characteristic of lepromatous leprosy susceptible individuals? And if so, what are the host (genetic) factors that drive this defect?

Whatever the issues to be resolved, the study by Krutzik, et al. sheds new light on Mf and DC in innate and adaptive immune responses in general and in leprosy in particular. Therapies to activate and expand DCs in lepromatous patients may help to control disseminating infection.

—Tom H. M. Ottenhoff, M.D., Ph.D., Michèl R. Klein

Dept. Immunohematology and Blood Transfusion
Leiden University Medical Center, Leiden, The Netherlands

Correspondence to: Tom H. M. Ottenhoff, M.D., Ph.D., Dept. Immunohematology and Blood Transfusion, Leiden University Medical Center, Albinusdreef 2 2333 ZA Leiden, The Netherlands, E-mail: t.h.m.ottenhoff@lumc.nl

REFERENCES
No country in the world can be more concerned about leprosy than India, as India represents nearly 76% of the global burden of the disease (1); India alone represents 87% of prevalence and 90% of new detected cases in the South East Asian Region. This clearly indicates that India is a key country in efforts to eliminate leprosy. (2)

The year 2005, which is the extended date for global elimination of leprosy, has brought new challenges to Indian leprosy workers and administrators. In their efforts to reach targets many steps are being taken by leprosy authorities of the Government of India, most of them with the support of the international agencies and NGOs. The Government of India, in its efforts to eliminate leprosy through its National leprosy elimination program (NLEP), has constantly been in consultation with organizations such as the World Health Organization (WHO), Global Alliance for Elimination of Leprosy (GAEL), and Non Governmental organizations (NGOs) such as the International Federation of Anti-Leprosy Associations (ILEP), and others.

India being the seventh largest and second most populated country in the world, the organization of leprosy services reaching to many distant parts of India was an enormous task. The WHO MDT program, which was initially introduced in India in 1983, could only be extended to all parts of the country by the end of 1995. (3) In the region of Jharkhand, for example, which is highly endemic for leprosy, MDT was introduced in only 1994–1995. (4) Thus, since the WHO in 1991 had declared its intention to reach the global elimination target by 2000, program managers were already planning for the elimination of leprosy even as the complete coverage of leprosy in all parts of India had barely been achieved, and infrastructure had just been put in place. As noted, however, this goal was extended to 2005 as WHO observed that 12 countries would not be able to achieve the elimination target by the year 2000.

Meanwhile, the MDT therapy in itself has undergone significant modifications with respect to the duration of therapy and also to the criteria for inclusion of patients into MDT-PB and MDT-MB groups. (5) Various methodologies were adopted in the leprosy program with the belief that they would benefit the patient and at the same time bring about rapid reduction in the prevalence rates of leprosy in India along with the rest of the world. Two such examples were the introduction the of single-dose ROM (rifampicin, ofloxacin and minocycline) therapy for single skin lesion leprosy (SSL –PB) (6) and initiation of the Leprosy Elimination Campaigns (LECs) all over India.

The rationale for ROM therapy was always controversial as it was based on a single multi-centric double blind field trial study whose results actually showed that ROM therapy was marginally less effective than MDT-PB in treating SSL-PB patients. (7) These results notwithstanding, ROM therapy was introduced in India probably because single skin lesions comprise a significantly high proportion (up to 60%) of leprosy patients in this part of the world. (8) For reasons not detailed, ROM therapy was dis-
continued five years after its introduction. However, the names of the patients who received ROM-single day therapy were removed from registers, as they were considered to have completed their treatment.

During the same time, Modified LECs (MLECs) were being conducted all over India and the state machinery participated with enthusiasm. There have been five MLECs between 1997 and 2004. Four nationwide MLECs have been conducted in the country as special efforts towards early detection of leprosy cases and their prompt treatment with MDT. The Fifth MLEC was conducted in eight high priority States during 2003–04.

In September 2003, with only one and half years to go to meet the deadline of leprosy elimination in India by 2005, the Government of India and the WHO organized a meeting in Goa of health secretaries of India’s major leprosy-endemic states. It was in part a follow-up to a meeting held in Tokyo in June, 2002. Several important NGOs also participated in this meeting. The Goa meeting recommended that the seven states in India where the prevalence rate of leprosy was between 1–2/10,000 would work hard to achieve the elimination target by March, 2005. These endemic states and union territories were advised carry out the Strategic Plan of Action discussed during the meeting in specified areas during the next one-year period. (8)

After the Goa recommendations, further meetings were held at various state headquarters and leprosy directorates of India to encourage these program officers to reach the elimination target by March, 2005. To do this, new instructions were given by the health authorities of Andhra Pradesh to the field staff. These instructions are called the “Kathmandu recommendations.” They are:

1. Stop all active search for case detection.
2. Do not register cases before reconfirmation by experienced staff.
3. Declare patients as released from treatment (RFT) and delete the names of these patients from registers as soon as they receive the last pulse of treatment.
4. Do not register single lesion cases for now.

The first three instructions were through official documents and office orders to the field workers. The last instruction was a verbal instruction; such verbal instructions were not limited to the state of Andhra Pradesh but were also given in other states of India.

Let us examine these directives. First, the first directive ‘to stop all active case detection’ is endorsed on the website of the WHO representative of India (2), which states that ‘at present, the emphasis for detection is based on routine voluntary reporting, with no more routine active case detection’. The WHO document containing the plan for leprosy work for the period 2006–2010 proposes the use of case detection as the main indicator to monitor progress. (10) It states that the important component of the leprosy control program is timely detection of new cases, but it recommends that case-finding should mainly be focused on promoting self-reporting.

With falling prevalence rates and the goal of elimination in sight, the focus of the leprosy program has shifted from an active search for new cases to voluntary reporting. However, there are some who feel that leprosy elimination cannot be accomplished without full geographic and population coverage and without intensified effort to treat all patients. (11) It is true that intensifying case detection may lead to over-registration and over-reporting of cases, but this should not mean that we do away with active searching for new cases. Although WHO evaluators observed that the number of new cases detected in LECs included a significant proportion of wrong diagnoses, re-registration, and ‘non-existent patients’ in programs, not all evaluators found LECs to be the cause of over diagnosis or re-registration. Some evaluation teams actually diagnosed additional new cases missed by the LEC teams. (13)

A balance needs to be struck between detecting all hidden cases and avoiding re-registration and wrong diagnoses. Intensive active case detection conducted through MLECs for the discovery of new cases proved to be one of the most successful health care interventions undertaken in India in recent years, particularly in the states of Bihar and Orissa. (14) It is only fair to say

that in a vast and diverse country like India, a combination of strategies and methods is required to reach varied target groups. (15)

The second guideline, which is re-validation by an experienced health worker within one month of the diagnosis of leprosy could serve to delay the inclusion of new patients into the registers and hence to help keep the monthly new case detection rate and prevalence rate within limits until the elimination goal is achieved.

The third guideline also serves a similar purpose, by deleting the patient from case registers and advancing the RFT date by a month. In such cases the last month’s therapy becomes accompanied MDT.

The fourth and important verbal instruction was confirmed with various health workers of Hyderabad, Andhra Pradesh. Issuance of similar verbal instructions was personally confirmed with health workers of one other state (Delhi) which is in the northern part of India, while Andhra Pradesh is in south India. It is not unreasonable to assume that such instructions may not be limited to these two states.

Single-lesion leprosy cases have always had a special place in leprosy. The percentage of single-lesion disease among leprosy patients in India is quite high. (7) Although single lesion leprosy is considered paucibacillary, multibacillary leprosy may also present as a single lesion. (16, 17)

Generally it is believed that single-lesion leprosy cases have no transmission potential and are not of great significance from the public health point of view, as a high percentage of these case show a tendency for self-healing. (18) However, large numbers of single-lesion cases detected represent an exposure of the population to a reservoir of infection which may contribute to the number of new cases and hence cannot be ignored. On the whole it is believed that at least a proportion of single-lesion leprosy will, without treatment, progress to multi-lesion leprosy. (19)

Some workers wanted single-lesion cases to be excluded from the number of leprosy patients when calculating new case detection rates, arguing that they do not contribute to the spread of the disease. (20) However, many studies have suggested that untreated MB patients do not represent the sole source of infection and that household contacts of PB patients have also been shown to be at a greater risk of developing the disease than non-contacts, although the risk is smaller than that of contacts of MB patients. (21) It is unwise and unethical to exclude single-lesion leprosy cases from the registers as new cases and to deny therapy. However, such non-registration/non-inclusion of single-lesion cases will substantially help the program managers to bring down the number of new cases and thus assist in reaching the elimination target in time.

What about the present leprosy statistics of Andhra Pradesh? The reported tentative average prevalence rate of leprosy in Andhra Pradesh state (with 22 districts) in mid 2004 (22) was 1.78/10,000, with 10 districts having rates of 1–2/10,000, 9 districts having rates of 2–3/10,000, and 2 districts with rates of 3–5/10,000. In the epidemiological indicators of new leprosy cases prepared for Andhra Pradesh up to March 2005, the percentage of child cases was 19.8% and of MB cases was 28.2%. Scheduled castes (SC) and Scheduled tribes (ST), who are the under-privileged of the society and live in areas with difficult access, constitute 33.5% of all new cases. A large proportion of children among the newly detected patients is a sign of active and recent transmission of infection, (21) especially when there is no active search or campaign for case detection. The large number of leprosy cases being detected among SC and ST populations indicates that focused health and communication campaigns are required to improve access to information and health services of these populations, particularly to those in remote areas.

In the neighboring state of Tamil Nadu, the leprosy prevalence rate was 1.4/10,000 in the year 2004. (23) On May 15th of 2005 the health ministry of Tamil Nadu has declared (24) that the present prevalence rate in Tamil Nadu is only 0.85/10,000, which means that it has already reached the elimination target. As other states are also encouraged to reach the targets, it is bound to happen sooner rather than later. It has already been reported in media that four southern states (Tamil Nadu, Andhra Pradesh, Kerala and Karnataka) have reached the elimination target of a prevalence rate of <1/10,000 population by May
of this year. (25) This was substantiated by a ‘news and notes’ report of a regional conference on leprosy held at Chennai, published by the Indian Journal of Leprosy. (26)

It will not be out of context here to consider the experience of the National Malaria Control Program of India, which was initiated in 1953—a story of failure. Initially it made rapid gains so that by 1961, the annual number of new cases registered was only 50,000. However, a resurgence of malaria was reported from 1962 onwards. By 1976, 6.4 million new cases were reported. Presently, the annual incidence is around 2 million. (27) Some of the important causes detailed for the failure of the National Malaria Eradication Program of India (28) were as follows: diversion of the work force, promoting newer priorities when greater effort was needed to root out the last pockets of endemicity, entrusting work to multi-purpose and basic health workers who were ill prepared for the task and, above all, laxity in national commitment and determination. It was also mentioned that the third world countries did not fully understand the epidemiological ‘rules of the game’. In short, the present resurgence of malaria is due to the relaxation of effort.

Similar indicators already exist in present leprosy program of India. Added to these are newer national priorities such as HIV and a resurgence of tuberculosis. Dilution and relaxation in the efforts of the NLEP has already set in.

What is being presented here is common knowledge in India, and the government orders cited were circulated openly and were not privileged information. Most of the NGO’s participating in NLEP in India would also be aware of these directives and figures, as they work very closely with the central and state governments of India and are participate in the national and international meetings and consultations. The credit for decreasing the leprosy prevalence and the efficacy of leprosy control program in India should be shared equally by GOI, WHO and NGOs. However, for reasons unknown, there seems to be a great hurry on the part of everyone involved with NLEP in India to reach the elimination target by the end of 2005 and to get on with a vision of leprosy beyond 2006.

—P. Narasimha Rao, Assistant Professor
D.V.S. Pratap, Professor and Head

Department of Dermatology and Leprosy,
Gandhi Medical College,
Secunderabad, Andhra Pradesh, India

Correspondence to: Dr. Rao, B-48, income tax colony, Mehdipatnam, Hyderabad – 500 028 India. E-mail: dermarao@hotmail.com

REFERENCES
2. WORLD HEALTH ORGANIZATION REPRESENTATIVE TO INDIA. http://www.whoindia.org/CDS/CD/leprosy/leprosy.htm accessed on 23rd June, 2005
6. Modified Guidelines on MDT Regimen to be Followed under NLEP. Directorate General (Lep), Nirman Bhavan, New Delhi, 1997
7. LOCKWOOD, D. N. Rifampicin/minocycline and ofloxacin (ROM) for single lesions—what is the evidence? Lepr. Rev. 68 (1997) 299–300


23. Health and Family Welfare Department, Government of Tamil Nadu http://www.tnhealth.org/dphdblep.htm accessed on 23rd June, 05


We are told that after 2005 we will enter a new, “post-elimination” era, during which leprosy cases will be rare. It is understandable that a new era requires a new strategy, which is why WHO/AFRO is circulating a strategy paper, which will be discussed at the AFRO annual meeting on leprosy, to be held from 27th to 29th June 2005 in Brazzaville, by the leprosy program managers of the WHO African region, and by the representatives of international non-governmental organizations. Nevertheless, the strategy is not really new; in fact, it does not differ substantially from the “Final Push” strategy. It remains “elimination”-oriented, and the quality of leprosy services continues to be ignored.

MY COMMENTS

1. The WHO/AFRO strategy misses a golden opportunity to re-define the priorities of leprosy control programs.

During the “elimination” era, the only priority was achieving the elimination target by bringing down the prevalence rate at any price. Hence, a number of simplified techniques for diagnosis and treatment of leprosy were implemented intensively, without paying adequate attention to quality control. At the same time, many essential activities — e.g., prevention of disability — were completely neglected in the field, primarily because these activities were unrelated to the prevalence. As a consequence, the quality of leprosy services was poor and achievement of the final goal of leprosy control was jeopardized. Now, in the “post-elimination” era, because political pressure to achieve the elimination target is diminished, the leprosy programs could, and should re-define the priorities of the activities, by focusing on quality of diagnosis and treatment and prevention of disability. Although the title of the strategy paper includes the phrase “to maintain the quality of leprosy services,” the relevant paragraphs in the text are extremely sloppy and vague, and fail to suggest concrete actions (see 5.2.2 and 5.2.3). “Prevention of disabilities” is mentioned only once (p. 3), but without details; one might therefore wonder how seriously the strategy deals with the issue of quality.

2. The strategy paper stubbornly upholds the poorly-justified technical policies that have already damaged the quality of leprosy services during the “elimination” era.

- The strategy paper over-estimates the sensitivity with which leprosy can be diagnosed using only clinical criteria, and under-estimates the important role of the skin-smear (see 5.2.1) in the diagnosis of smear-positive MB leprosy patients and relapsed MB patients, who represent the major sources of leprosy infection in the community. Apparently, the authors of the strategy paper do not understand that a significant proportion of smear-positive MB patients (especially those close to the lepromatous end of the spectrum), and the great majority of relapsed MB patients cannot be diagnosed without skin-smears; the strategy paper therefore fails to recommend re-introduction of skin-smear service in the field.
- Supervised administration (or directly observed treatment) of the monthly
component of MDT regimens is an important element of the multi-drug therapy for leprosy, which ensures that the patients take the right drugs, in the right doses, at the right intervals. However, the strategy paper continues to ignore the supervised administration of the monthly component of MDT regimens by promoting “flexible MDT,” especially so-called “accompanied MDT” or “self-supervision” (which is, in fact, no supervision) (see 5.2.8). The recommendation that the patient who has received the total amount of MDT drugs at the beginning of treatment and “who is not seen in a health facility at the end of his treatment should be considered as having no concern on his condition and being cured” (original phrase in 5.2.8) is ridiculous; it is virtually the same as declaring that the patient is cured at the time he receives the total amount of MDT drugs.

• Relapse and emergence of drug resistance are the most serious outcomes of poor treatment in any large-scale treatment campaign including MDT for leprosy, and all efforts should therefore be made to prevent or reduce their occurrence. Surprisingly, the strategy paper omits any mention of detection and prevention of relapse after MDT and emergence of rifampicin-resistant leprosy, as if these phenomena have not been encountered and will not occur; such a blindly optimistic attitude is an invitation to disaster.

• As already mentioned, “prevention of disability” is grossly neglected.

MY RECOMMENDATIONS

1. The strategy paper should be thoroughly revised. Involvement in the revision of the program managers and representatives of NGOs and scientific community is highly desirable.

2. The priority of leprosy control activity should focus on quality and sustainability of leprosy services, especially in the areas of diagnosis, treatment and prevention of disability.

3. The efficiency of integration and decentralisation should be reviewed.

4. The potential role of general health workers in case-finding and case-management should be reviewed and, possibly, revised. When leprosy cases become rare, it would be more logical that general health workers at the most peripheral level be responsible only to detect suspected cases; the diagnosis of leprosy will be validated or confirmed by more experienced workers from either the district or the referral center.

5. Serious efforts should be made to increase the number and improve the quality of the referral centers; ideally, each endemic district will have one. The role of these centers should be defined in detail.

6. Training of health workers should be an important component of the strategy. With support from NGOs and other partners, AFRO should provide assistance to train the trainers for each of the national leprosy programs. At the country level, basic training must be provided to those workers responsible for the leprosy program at the national, intermediate and district levels, to make certain that they are able to manage the program and deal with patients independently; for those workers at the most peripheral level, training is still necessary but needs only to be task-oriented.

7. The recommendation that leprosy might be diagnosed by the presence of anaesthetic skin lesions alone is problematic, because about 30 per cent of leprosy lesions are non-anaesthetic, and most of these are observed in smear-positive MB cases. To improve the quality of diagnosis, instead of relying upon a single criterion, leprosy should be diagnosed by presence of one or more of the three cardinal signs (anaesthetic skin lesions, thickened peripheral nerves, and acid-fast bacilli in the skin-smear or biopsy specimen). Diagnosis of leprosy will mainly be the responsibility of health workers who have received better training and have access to skin-smears, presumably at the district level or at the referral centers.

8. The skin-smear service must be reintroduced in the field, beginning in leprosy endemic areas; the skin-smear service may be associated or combined with the laboratory facilities of the tuberculosis program.

9. To improve the quality of MDT treatment, adherence of patients to treatment should never be compromised; therefore, supervised administration of the monthly component of MDT must be ensured. The
supervisor could be one of the staff in the health facility; for those patients who may have difficulty to visit the health facility once monthly, the supervisor could be a community health worker or a trained local community member. In general, members of the patient’s family should not serve as treatment supervisor.

10. To detect relapse after MDT and the emergence of rifampicin-resistance, post-MDT surveillance should be reintroduced, and skin-smear positive MB patients should be examined both clinically and microscopically (skin-smears) once yearly for as long as 7 years after completion of MDT. AFRÓ should identify the facilities that are capable of testing the rifampicin-susceptibility of the relapsed strains detected by the programs.

11. Because the prevention of disability has been neglected for too long, the national leprosy program should make special efforts to initiate this activity, including training of health workers, health education of the patients and the community, identification and upgrading of the referral centers, supplying medications, and providing social and financial support to the patients when necessary.

12. Community participation in leprosy control activities should be encouraged, especially in the areas of case-finding, case-holding, prevention of disability and social rehabilitation.

—Baohong Ji, M.D.

Association Française Raoul Follereau, Paris, France
TO THE EDITOR:

In 1998, we had suggested the quantitative method of sensory assessment of face and testing sites for the limbs (1). The following improvement to the original method may be required and this is based on our clinical experience in using this technique in a referral center with specialists and time available.

The 10 sites for testing sensation on the face, hands, and feet are unchanged. We suggest two changes with the Semmes-Weinstein monofilaments as a result of the recent understanding of the normal sensation of the hands and feet. Similarly, the method to score sensory nerve status is also altered. This is because the clinicians expressed that the norms for muscle grading are: “zero” indicating flaccidity, and a maximum score of 5 given for normal musculature; this was reversed in our quantitative sensory testing. In order to have a uniformity between sensory and muscle testing, we recommend the changes depicted in the following assessment form. In the revised form, 0 to 4 sensory grading system is followed for the hands. For the foot, 0 to 3 grading system is used because their sensory function is less than that of hands, which have to manipulate objects and require well developed sensory nerve endings. For the face, a 0 to 3 grade sensory threshold scale was used with the interpretations suggested by Premkumar, et al. (4).

The interpretations presented for the foot and hand are also based on the following previous scientific studies. Krotoski published the details on interpretation for the hands (1). Similarly, Birke, et al. interpreted 10 g filament as the level of protective sensation in leprosy patients (2). Kets, et al. study demonstrated that the touch sensibility monofilament threshold screening in healthy Nepalese population were 0.2 g for hands and 2 g for feet (3). Since all of the South Asian population is likely to be similar to that of Nepalese, we had taken the interpretation of this study and made a small modification to Krotoski’s hand sensory battery by removing 0.05 to 0.07 g filament as an instrument to test normal sensation. In the original neurological mappings by Weinstein demonstrated the higher sensitivity in the face; the mean threshold of males to be 0.02 g; females, 0.018 g (5). Despite the above work in neurology, in the facial sensation assessment we suggest using a filament that gives a force of 0.05 to 0.07 g. It will be higher than the threshold for the face and will avoid false negative responses for the following reason: The lowest sensory threshold in normal individuals quoted in the Weinstein article is in the laboratory situation, which cannot be duplicated in clinics. Therefore, the next higher threshold may be required to increase the test sensitivity.

We are also aware that more studies are needed to answer the following research questions arising from this work. For instance, the lack of testing the corneal sensation to an extent limits the usefulness of testing facial sensation. Since this study

Quantitative Measurement of Sensory Impairment in Referral Centers¹

¹This article is a revised version of the original published in the International Journal of Leprosy, Volume 73, Number 3, 2005.
Summary Table.

<table>
<thead>
<tr>
<th>Body Part</th>
<th>Nerves</th>
<th>Number of Sites Tested (per nerve)</th>
<th>Maximum For Each Right</th>
<th>Score Nerve Left</th>
<th>Maximum Score For Each Body Part</th>
</tr>
</thead>
<tbody>
<tr>
<td>Face</td>
<td>Trigeminal</td>
<td>3</td>
<td>/9</td>
<td>/9</td>
<td>/18</td>
</tr>
<tr>
<td></td>
<td>Auricular</td>
<td>2</td>
<td>/6</td>
<td>/6</td>
<td>/12</td>
</tr>
<tr>
<td>Hands</td>
<td>Ulnar</td>
<td>4</td>
<td>/16</td>
<td>/16</td>
<td>/40</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>6</td>
<td>/24</td>
<td>/24</td>
<td></td>
</tr>
<tr>
<td>Foot</td>
<td>Posterior Tibial</td>
<td>10</td>
<td>/30</td>
<td>/30</td>
<td>/30</td>
</tr>
</tbody>
</table>

"Zero" score indicates maximum sensory loss. The denominator indicates normal sensation.

Correspondence

KEY FOR GRADING

<table>
<thead>
<tr>
<th>Face</th>
<th>Not Felt</th>
<th>Felt</th>
<th>Interpretation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 g</td>
<td>0.2 g</td>
<td>Normal superficial sensation</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0.2 g</td>
<td>2.0 g</td>
<td>Normal superficial sensation diminished</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2.0 g</td>
<td></td>
<td>Loss of normal superficial sensation—deep sensation intact</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.0 g</td>
<td>Total loss of pressure sensation</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Foot</th>
<th>Not Felt</th>
<th>Felt</th>
<th>Interpretation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 g</td>
<td>10 g</td>
<td>Normal superficial sensation</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>10 g</td>
<td>300 g</td>
<td>Normal superficial sensation lost—protective sensation intact</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>300 g</td>
<td></td>
<td>Protective sensation lost—deep pressure sensation intact</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 g</td>
<td>Total loss of pressure sensation</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hand</th>
<th>Not Felt</th>
<th>Felt</th>
<th>Interpretation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2 g</td>
<td></td>
<td>Normal superficial sensation</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2 g</td>
<td>4 g</td>
<td>Normal superficial sensation diminished</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>4 g</td>
<td>300 g</td>
<td>Superficial sensation lost—protective sensation intact</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>300 g</td>
<td></td>
<td>Protective sensation lost—deep pressure sensation intact</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>300 g</td>
<td>Total loss of pressure sensation</td>
<td>0</td>
</tr>
</tbody>
</table>

| Summary Table. |

<table>
<thead>
<tr>
<th>Body Part</th>
<th>Nerves</th>
<th>Number of Sites Tested (per nerve)</th>
<th>Maximum Score For Each Nerve</th>
<th>Maximum Score For Each Body Part</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Face</td>
<td>Trigeminal</td>
<td>3</td>
<td>/9</td>
<td>/9</td>
</tr>
<tr>
<td></td>
<td>Auricular</td>
<td>2</td>
<td>/6</td>
<td>/6</td>
</tr>
<tr>
<td>Hands</td>
<td>Ulnar</td>
<td>4</td>
<td>/16</td>
<td>/16</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>6</td>
<td>/24</td>
<td>/24</td>
</tr>
<tr>
<td>Foot</td>
<td>Posterior Tibial</td>
<td>10</td>
<td>/30</td>
<td>/30</td>
</tr>
</tbody>
</table>

“Zero” score indicates maximum sensory loss. The denominator indicates normal sensation.


confines in using the instrument of S-W filaments in testing only the skin in the limbs and face, and not the cornea, it is beyond the scope of this work. In the previous work on facial sensory testing, the authors hypothesized that the corneal sensation assesses only the ophthalmic branch of the trigeminal nerve (4). The other two branches of the nerve usually go unexamined. Facial sensory testing, we have suggested, will give quantitative sensory information for all three branches of the trigeminal nerve. Hence, the specific research question would be whether testing the facial sensation around the eyes could indicate corneal insensitivity?

There is also a research question related to the testing sites: whether further reduction in the number of testing points would be more beneficial than the 25 sites we proposed? Our suggestion for further testing sites reduction is to 10; for example, two each for facial, great auricular, ulnar, median and posterior tibial. A further scrutiny is also needed into the validity of the facial sensory loss and its interpretation to function that we have suggested in our previous work (4), in a larger population.

Method used to score sensory nerves supplying face, hand and feet

Ten testing sites have been selected for each hand, foot and face. Three testing points have been identified for each trigeminal and 2 for each great auricular nerve: 4 for ulnar, 6 for median and 10 for posterior tibial. If the patient feels 0.05 g filaments in the face and 0.2 g in hands or 2 g filaments in
Serologic Recognition of Low Molecular Weight Mycobacterial Protein Fractions in Lepromatous Patients with Type II Reactions (ENL)

TO THE EDITOR:

Hansen’s disease is a mycobacterial infection that produces physical disabilities. The progression of the disease is slow and indolent but in some cases there are changes in the immunological status with the development of acute episodes represented by reactional states. Many of these reactional episodes occur after treatment has been finalized and, therefore, it is important to clarify whether they constitute relapses. We wished to determine if specific patterns of serologic recognition of mycobacterial proteins were associated with Type 2 reactional states in lepromatous patients. Serum samples were taken from 12 adult patients, mean age of 43 ± 16 yrs, with a predominance of males (80% M and 20% F), who were undergoing a Type 2 reactional episode (erythema, nodosum leprosum, ENL). These sera were divided in two groups of six sera each: sera

---

— Ramaswamy Premkumar, Ph.D., Pichaimuthu Rajan, BOT, Ebenezer Daniel, MS, MPH

Schieffelin Leprosy Research and Training Centre, Karigiri - 632106, Tamil Nadu, India.

REFERENCES
from Group I were antibody-positive to phenolic glycolipid (PGL-I), the other six (Group II) were negative. ENL reactions were characterized using histopathological criteria, including the presence of undifferentiated macrophages and relatively abundant PMNs, with or without acid-fast bacilli. The group of six patients that gave negative reactions for antibodies to PGL-I (Group II) had completed multidrug therapy; they presented an average of six episodes of ENL. Of the six patients in Group I with detectable antibodies to PGL-I, two were still being treated. ENL reactions were less frequent in Group I (average 4 episodes).

Soluble component fractions were obtained by an electroelution technique from *Mycobacterium leprae* soluble extract (MLSA) and *Mycobacterium bovis* soluble extract (MbSA) (5, 6). The soluble extracts were obtained by rupturing purified bacilli with the French Press (7). The extracts contain cytosol proteins as well as proteins freed from the cell walls. Insoluble material was eliminated by centrifugation. Protein concentration was determined by the BCA method (7).

Starting with a 10% SDS-PAGE preparative gel under dissociating and denaturing conditions, 1 mg of MLSA and MbSA was resolved in polypeptides of different mobilities (see The Figure), which were fractionated by electroelution in a mini BIORAD® 65-1256 electroelutor, according to the instructions provided by the manufacturer.

Twelve electroeluted fractions were obtained for both the MLSA and the MbSA antigens. ELISA tests were used to evaluate activity with the pooled sera, using IgG antibodies specific for the Fc gamma chain (Sigma A0170) as the second antibody (4).

A clear difference in recognition was seen between the two groups of sera studied. In the ELISA tests with both MLSA and MbSA electroeluted fractions, we saw an immunodominant recognition of proteins with a relative mobility of 30 kDa, corresponding to Fraction 9 (see The Table). There was also serologic recogni-

---

**THE TABLE.**

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Fractions MbSA</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td></td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>0.241 ± 0.001</td>
<td>0.470 ± 0.01</td>
<td>0.256 ± 0.01</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
</tr>
<tr>
<td>GII</td>
<td></td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>0.296 ± 0.003</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patients group</th>
<th>Fractions MLSA</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
<th>F9</th>
<th>F10</th>
<th>F11</th>
<th>F12</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI</td>
<td></td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>0.257 ± 0.004</td>
<td>0.501 ± 0.001</td>
<td>&lt;0.125</td>
<td>0.313 ± 0.03</td>
<td>&lt;0.125</td>
</tr>
<tr>
<td>GII</td>
<td></td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>0.178 ± 0.025</td>
<td>0.483 ± 0.01</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
<td>&lt;0.125</td>
</tr>
</tbody>
</table>

The results are expressed as optical density (O.D.) at 492 nm. To establish the criterion of positivity, the value resulting from mean OD plus 3 times the standard deviation of 12 healthy subjects was used as the cut-off point (0.125 O.D. units).
tion of low molecular weight MLSA proteins (less than 30 kDa) in patients in group I which was not observed in Group II.

In preliminary studies we previously reported a clear difference between the IgG antibody levels directed towards soluble mycobacterial proteins (Mycobacterium bovis MbSA and Mycobacterium leprae MLSA) in an ENL active group (n = 4) as compared with the non-active group (n = 4) (3). In the ENL active patients we found IgG antibody levels towards MbSA and MLSA of 0.535 ± 0.24 and 0.731 ± 0.32, respectively, as compared with the non-active patients, whose values towards the same total proteins were zero. In this study using the electroelution technique we were able to demonstrate the immunodominant antigens found in patients in an ENL reactional state.

Many authors have shown a decrease of IgM antibodies directed towards phenolic glycolipid (PGL-I), which is an M. leprae structural component (1) in these reactional patients. To examine this, we separated the reactional patients in two groups, according to their PGL-I positivity. IgM antibodies against native PGL-I were measured in an enzyme linked immunosorbent assay using the method described previously (8).

In addition to the immunodominant recognition towards proteins with a 30 kDa relative mobility, both with MbSA and MLSA, we also saw that the recognition in Group I involves a larger number of protein fractions, including low molecular weight proteins (<30 kDa), compared to the patients in Group II.

We have recently increased the number of multibacillary patients (n = 70), and there have been no significant differences in the Mycobacterium leprae 30 kDa protein antibodies between patients who had Type II reactions and those who did not. In this larger group of 70 multibacillary patients, nine presented ENL reactions and the other 61 did not. Of the nine with ENL, eight (89%) gave positive reactions to the 30 kDa protein, average optical density 0.8816. Of the 61 remaining patients, 42 (69%) gave positive reactions to the 30 kDa protein, average OD 0.5885. This difference was not statistically significant, p = 0.42, but the observation suggests a trend toward stronger reactivity in patients with ENL. The sera of newly diagnosed multibacillary patients reacted with other peptides of both higher and lower molecular weights. In this population of 70 patients, 62.6% were in treatment and presented bacillary indices of less than 2+. Reactivity was strongly associated with bacillary load. Reactivity to the 10 kDa protein of M. leprae was lower in treated patients than in new cases (unpublished data).

In conclusion, both patients who had ENL as well as those who did not responded to the 30 kDa peptide of M. leprae, but the reactions tended to be stronger in the former group. Additional more detailed studies will be necessary to detect a clear marker for ENL, using individual proteins of the 85B complex or specific peptide sequences of other proteins that might discriminate between patients with or without reactional phenomena.

**Acknowledgment.** This research was supported by grant number S1-2001000859 from Fondo Nacional Ciencias y Tecnología (FONACIT) Caracas, Venezuela.

—Elsa María Rada, M.Sc.,
**Laboratorio Leprología y Patología Experimental**
**Instituto de Biomedicina,**
**Caracas, Venezuela**

—Edgar A. Zambrano, Ph.D.,
**Laboratorio de Bioquímica de Parásitos,**
**Instituto de Biomedicina,**
**Caracas, Venezuela**

—Nacarid Aranzazu, M.D.,
**Clinical Section M.D., Instituto de Biomedicina**
**Caracas, Venezuela**

—Jacinto Convit, M.D.,
**Director, Instituto de Biomedicina**
**Caracas, Venezuela**

**REFERENCES**

2. Convit, J., Aranzazu, N., Ulrich, M., Pinardi, M. E., Reyes, O., and Alvarado, J. Immunotherapy with a mixture of Mycobacterium leprae and...
Paucibacillary Treatment for Large Tuberculoid Lesions of Leprosy?

TO THE EDITOR:

Under the title “Should large lesions of leprosy be considered as multibacillary for treatment purposes even if the total number of lesions is less than five?” [Int. J. Lepr. 72 (2004) 173–174], Kumarasinghe and Kumarasinghe called attention to an interesting aspect regarding the treatment of big size tuberculoid lesions or borderline-tuberculoid lesions according to Ridley & Jopling classification.

Their arguments for the treatment of patients with large plaques are valid but the recommendation since the beginning of Multi-drug Therapy - M.D.T./World Health Organization/82 (2) was to treat patients upon a positive or negative bacilloscopy. The size or the number of lesions were not to be taken into account. Millions of patients have been treated since, with a relapse rate of less than 1%. We present a patient classified and treated as PB leprosy with a large plaque and five smaller lesions.

**Patient.** N.R.L, 45 years old, registered at the Fundação de Medicina Tropical do Amazonas, Manaus, Brazil.

The patient presented a large plaque lesion on the chest (Fig. 1) and five smaller lesions on the face, arm and posterior part of the trunk. No enlargement of the ulnar nerve or of other peripheral nerves could be found. The patient was clinically classified as reactional borderline tuberculoid leprosy.

The histopathology (Hematoxylin-Eosin) showed a granulomatous lesion with lymphocytes, histiocytes and giant cells (Fig. 2). The Wade stain was negative for acid-fast bacilli. The patient was classified as borderline tuberculoid (BT) leprosy.

Paucibacillary M.D.T. according to the W.H.O, plus 60mg prednisone per day was started in March 2003. M.D.T. was stopped after 6 months of regular treatment and cortisone was slowly tapered off after 3 to 4 months.

All the lesions regressed leaving a hypopigmented area (Fig. 3). During the last clinical reexamination (10/11/04) no relapse was found. A new histopathology of the edge of the lesion showed a regressive infiltrate (Fig. 4).

**COMMENTS**

The W.H.O. recommendation to treat leprosy was based on bacilloscopy for many years on the bacilloscopy results, and the efficacy of M.D.T. has been the same worldwide: Less than 1% of relapses. The W.H.O. (3) recommendation to treat leprosy patients according to the number of lesions was mainly an operational decision to implement M.D.T. in the field. There was no recommendation related to the size of the lesion.

We think that a patient with a negative bacilloscopy and a histopathology consistent with reactions bordering the tuberculoid category can be treated after clinical and histopathological evidence, with the same efficacy of the bacillary patients.
with a tuberculoid granuloma with scarce or no bacilli in the Wade or Fite-Faraco stain must be classified as paucibacillary leprosy. We agree with Kumarsinghe and Kumarsinghe (1) that “. . . the larger the lesions of leprosy, the higher the number of bacilli that cause the pathology . . . ,” but in such a lesion the clinical aspect is roughly the same with defined edges and very well established borders between the lesion and the normal skin. Besides the relatively uniform clinical aspect as observed in our patient, the skin smears and the histopathology were the same in repeated biopsies with the number of bacilli scarce or negative. We could not find any information in the literature to substantiate the statement of the authors, that tuberculoid leprosy could evolve to the lepromatous pole over several years with the lowering of patient’s cellular immunity (1). We have been treating patients with multiple (more than 5) lesions as PB leprosy when the clinical aspect of the lesions and the histopathology showed a picture of tuberculoid leprosy.

We agree with Kumarasinghe and Kumarasinghe (1) that the W.H.O. recommendation is “particularly important in areas where treatment is initiated without any bacteriological and histopathological confirmation . . . .” However, in referral centers and in universities with good laboratory support the present WHO guidelines to treat as PB leprosy or MB leprosy should not be followed. It seems there are no scientific data to justify a formal recommendation to treat leprosy according to the number or size of the lesions.

Acknowledgement. To Dr. Gotfried Schmer of the State University of Washington.
Drs. Kumarasinghe Reply: Should Large Lesions of Leprosy Be Considered as “Multibacillary” for Treatment Purposes Even If the Total Number of Lesions Is Less Than Five?

TO THE EDITOR:

We thank Souza Santos, et al. for their interest in our article (5). While agreeing with some points made by them, it seems that they have misunderstood some of the points we made.

First, our recommendation of “considering large lesions of leprosy as multibacillary” was not aimed for teaching hospital settings where microbiological and histopathological facilities and good clinical expertise are available, but for field settings, in areas where treatment is initiated without any investigations, purely based on the number of hypopigmented lesions. At the teaching hospitals and tertiary care centers we also treat patients after considering the smear results and skin biopsy results, in addition to the clinical picture.

It is well known that even a single lesion can be multibacillary (3, 4, 6). The rationale of total number of lesions as the only criterion for deciding on the treatment type, as well as for scientific analyses has been questioned (7). However, in a retrospective study carried out in India, Gift, et al. have found that World Health Organization (W.H.O.) operational classification is a satisfactory method for deciding on the form of treatment (2). In this analysis, they have taken the smear examination as the gold standard for evaluating the sensitivity and specificity of the W.H.O. operational classification. However, where the larger lesions (>10 cm) were present they have found that the specificity was 91.2% although the sensitivity was low. As only 4.9% of smear positive cases have had records of the size of the lesion, that study appears to be inadequate to evaluate the validity of the size of lesions as an additional parameter.

Our recommendation for treatment of large plaque leprosy with three drugs for one year (“multibacillary treatment”) is based on the observation of more relapses in this group of patients who have been treated with two drugs for six months. In another study conducted in Sri Lanka it was shown that several patients with large lesions (>10cm) of leprosy were smear positive although the total number of the patches was less than five (1).
We do not dispute that many cases of paucibacillary leprosy have less than 5 patches. Although the authors agree on the W.H.O. operational classification based on the number of patches, the case described by the authors, with a large plaque of leprosy plus 5 other lesions on the face, would have been classified as “multibacillary”, if the microbiological investigations were not done, going by the visual classification recommended by the W.H.O. It is known that some cases of leprosy may improve even with dapsone monotherapy (as was the practice before the advent of multi-drug therapy, M.D.T.), or single dose multidrug therapy. It would be interesting to see the long term outcome of the case presented by the authors. Even though a smear was negative, in the case presented by the authors, we would have not have been comfortable in administering paucibacillary treatment only for 6 months, in a patient with such extensive lesions. Cell mediated immunity is of paramount importance in the pathogenesis of leprosy (7). It is clear that patients progress in the leprosy spectrum towards the lepromatous pole when the immunity of the host is unable to overcome the infection by lepra bacilli. In cases of subpolar lepromatous leprosy some areas with typical hypopigmented semianesthetic lesions can often be seen while other smear positive lesions coexist in the same patient. Clearly not all cases of multibacillary leprosy start as polar lepromatous leprosy. In our statement in the article we did not imply that “polar tuberculoid leprosy” would down-grade to “polar lepromatous leprosy” which are generally immunologically stable.

We agree with the authors that a larger scale study would be helpful to resolve the issue whether larger lesions due to leprosy should be treated with the “multibacillary drug regime” at least for one year.

A representative lesion should be microbiologically and histopathologically evaluated whenever possible, and the findings should be evaluated in conjunction with the clinical features before commencing on treatment. The current W.H.O. operational classification; while being useful in the community perspective, appears to be an over-simplification in some situations. The search for any additional features to fine tune the parameters should be continued.

—S. Prasad W. Kumarasinghe

Senior Consultant Dermatologist,
National Skin Centre, Singapore

—M. P. Kumarasinghe

Senior Consultant Pathologist,
Singapore General Hospital, Singapore

REFERENCES


1 Reprint requests to: S. Prasad W. Kumarasinghe.
MBBS, MD, FCCP, FAMS, Senior consultant Dermatologist, 1 Mandalay Road, National Skin Centre, Singapore. E-mail: prasadkumarasinghe@yahoo.com
Has the Term “Elimination” Outlived Its Utility?

TO THE EDITOR:

Please permit us to make some more observations (arising from our combined experience of over 60 years in leprosy relief work) particularly relevant to India, which contributes 77% of active cases to the global pool of active leprosy cases. One to 1.5 million out of 2 to 3 million leprosy-disabled in the world are reported to live in India.

Dr. Yo Yuasa, who was the President of the International Leprosy Association for two terms, exhorted everyone to work towards a “World Without Leprosy” at the International Leprosy Congress, Beijing in 1998. He defined this state as “a world without leprosy-related problems, both medical and social, emphasizing the point that it is not the disease per se but its related problems, mostly social but some medical, which require attention.”

This slogan was, however, pooh-poohed by the World Health Organization (W.H.O.) and the W.H.O.-influenced governments and the “program managers,” who were obsessed with the term “Elimination.” The target year was 2000, which is now revised to 2005, when the mean prevalence rate of 1 case per 10,000 is expected to be reached. Unfortunately by then, the world will also be free from the so-called “Leprologists.” The enormous funds still needed to do justice to the clinical problems related to leprosy and the rehabilitation of patients would have dried up. The “pool” of leprosy patients with reaction, neuritis and its sequelae, and those needing rehabilitation contributing to the “disease burden” in the community will far out number the active cases needing multi-drug therapy (M.D.T.) As yet there is no evidence of the much talked about secondary level and tertiary level “Referral Centers” easily accessible to patients living in areas deprived of even basic health services, where the primary health centers with which leprosy is “integrated.” Most patients and the health providers are not even aware of the technology to prevent the adverse progression of complications and palliative care of irreversible disabilities, let alone the concept of “Community-Based Rehabilitation.”

It is strange that the same public health specialists who talk about “Elimination” have now started fighting for “Human Rights” of leprosy patients without even attempting to formulate a mass-based strategy for addressing the clinical problems of patients “released from control.”

Perhaps they are waiting to celebrate the eventful day of 31 December 2005 to announce their “Victory over Leprosy” before thinking of planning the secondary and tertiary level referral centers! It is time that the people, patients, and particularly the donors are made aware that this victory is by no means a victory over all leprosy-related problems, as enshrined in the definition of “World Without Leprosy.” The donors are made to believe that with the magic word “Elimination,” the disease is already on the verge of being wiped out.

Has not the jargon “Elimination” of leprosy outlived its utility? Though it is rather late, should we not devise a more patient-friendly term for “Elimination” that truly reflects the sincere attempt at the eradication of all ills afflicting the persons who have contracted specially the progressive forms of the disease?

—Dr. R. Ganapati,
—Dr. V. V. Pai

Bombay Leprosy Project
The 40th Anniversary Meeting of all of the panels of the US-Japan Cooperative Medical Sciences Program (USJCMSP) took place in Kyoto in December, 2004. The Joint Tuberculosis and Leprosy Panels organized two half-day sessions dedicated to TB and leprosy, and co-sponsored two half-day sessions with other panels—one with the AIDS Panel on TB/HIV interactions, and the other with the Acute Respiratory Infections Panel on antibiotic resistance. The Joint Committee of the USJCMSP announced that new guidelines for panel activities would be implemented in the coming year. The Joint Tuberculosis and Leprosy Panels were encouraged to develop high priority scientific programmatic goals, and identify implementable research objectives for the next five years. Examples might include TB vaccine and drug development, management of latent TB, and the development of molecular tools to better characterize leprosy transmission and incidence. A strong emphasis will be placed on strengthening the research capacity for both diseases in high-burden countries in the Pacific Rim, including training activities and technology transfer. The Joint TB and Leprosy Panels were also encouraged to develop strategies for interacting effectively with other relevant Panels in the coming years.

The 40th Annual US-Japan Conference on Tuberculosis and Leprosy will take place in Seattle from 28–30 July, 2005. The main conference will be preceded by a half-day session co-sponsored with the Immunology Board which will focus on the definition of immunological determinants of protection induced by TB vaccines. The final day of the conference will consist of a Leprosy Workshop with invited speakers which will focus on the immunology and pathology of the disease, animal models, and challenges in leprosy research.

Modulation of the TH1 response to Mycobacterium leprae in experimental leprosy

Introduction. In leprosy, a disease caused by the obligate intracellular pathogen, Mycobacterium leprae, an array of symptoms are presented which are largely determined by the host’s response, ranging from a high level of cell mediated immunity (CMI) in tuberculoid leprosy (TT) to absence of CMI in lepromatous leprosy (LL). Animal models for leprosy are limited. Armadillos exhibit a disease spectrum similar to man, but they are restrictively expensive and immunological reagents are scarce. The murine system, while well-characterized and armed with a plethora of immunological reagents, is essentially restricted to the foot pad for evaluating growth of M. leprae and exhibits limited nerve involvement by the bacilli. Nevertheless, murine models for leprosy, especially with the introduction of gene knockout (KO) strains, have shown promise for immunological studies of the leprosy spectrum. M. leprae-induced granuloma formation and maintenance depends heavily upon T cell and macrophage (MΦ) populations and their respective cytokines. Mice deficient in inducible nitric oxide synthase (iNOS), an important MΦ effector mechanism, have shown promise as a model for borderline tuberculoid leprosy in that intense granuloma formation rapidly appears without exacerbating M. leprae growth. IL-12, a key regulatory cytokine of the immune system, induces the production of IFN-γ by T cells and NK cells and promotes the development of a Th1 type cell mediated immune response. IL-10 is generated by T cells and MΦ and is an inhibitor of IFN-γ production.

Methods. M. leprae infection was evaluated in iNOS KO (NOS2–/–), IL-10 KO (IL10–/–) and IL-12 KO (IL12–/–) mice using low dose (LD) and high dose (HD) infection models. C57Bl/6 (B6) control mice and
KO mice were infected in both hind foot pads with $6 \times 10^3$ (LD) viable *M. leprae* and growth, lymph node cell profiles and histology were monitored for 18 months. B6 and KO mice were also inoculated with $3 \times 10^7$ (HD) viable *M. leprae* in both hind footpads, with or without treatment with iNOS inhibitors such as LNil (L-N6-(1-iminoethyl) lysine hydrochloride) or aminoguanidine (AG). Foot pad induration, cell profiles, and cytokine expression were analyzed in these foot pads.

**Results.** In the LD model, growth of *M. leprae* was controlled in the NOS2$^{-/-}$ and IL10$^{-/-}$ mice, similarly to B6 control mice. In contrast, there was augmented growth of the bacilli in the IL12$^{-/-}$ mice. Flow cytometric analysis of the draining popliteal lymph nodes showed a sharp decrease in the level of T lymphocytes in B6 mice at 6 months post infection which corresponds with the peak of *M. leprae* growth. A similar decrease was seen in IL10$^{-/-}$ mice. In contrast, this decrease was not seen in IL12$^{-/-}$ mice. In the HD model, a large granulomatous response occurred in the NOS2$^{-/-}$ mice compared to B6 mice which consisted primarily of CD11b$^+$ MΦ and CD4$^+$ lymphocytes and, to a smaller extent, CD8$^+$ cells. The level of CD4$^+$ and CD8$^+$ cells expressing activation markers was significantly higher in NOS2$^{-/-}$ mice than B6 mice. Concomitant with foot pad induration was an augmented expression of IFN$\gamma$, TNF$\alpha$, and IL-10 as well as MIP-1$\alpha$, MIP-1$\beta$, and MCP-1. A similar induration occurred in *M. leprae*-infected B6 mice treated with L-NIL. Interestingly, the induration subsided if the iNOS inhibitor was removed; conversely, if the iNOS inhibitor was added 1 month post infection, enhanced induration ensued, thus emphasizing the dynamic nature of the foot pad lesion. Upon infection with HD *M. leprae*, IL10$^{-/-}$ mice also exhibited greater induration than control mice. Like B6 and iNOS$^{-/-}$ mice, the T lymphocyte infiltration was primarily CD4$^+$. Addition of LNil to the IL10$^{-/-}$ diet resulted in greater induration of the *M. leprae*-infected foot pad than either individual model of deficiency. If LNil administration began 1 month post infection, induration rapidly exceeded that of the IL10$^{-/-}$ mice and was similar to mice that were iNOS and IL10 deficient from the beginning of infection. In IL12$^{-/-}$ mice, HD infection with *M. leprae* induced little induration in the foot pad and the T lymphocyte infiltration was equally CD4$^+$ and CD8$^+$.

**Discussion.** These findings suggest that KO mice infected with *M. leprae* can provide insight into the subtle nuances of cell mediated immunity toward *M. leprae* infections as well as contribute to the overall understanding of the various processes underlying the broad host response to infection and, in particular, the unstable nature of the borderline area of the leprosy spectrum.

—Deanna A. Hagge, Nashone A. Ray, Vilma Tulagan and Linda B. Adams

**National Hansen’s Disease Programs, USA.**

**Initiative for Diagnostic and Epidemiological Assays for Leprosy (IDEAL)**

In 1977, the World Health Organization (WHO) Expert Committee on Leprosy estimated the global number of leprosy cases to be over 12 million. In 1981, WHO convened the Study Group on Chemotherapy for Leprosy Control, which recommended combined-drug regimens based on supervised intermittent administration of rifampicin for both multibacillary (MB) and paucibacillary (PB) leprosy. Thanks to the implementation of this multidrug therapy (MDT), substantial progress in leprosy control has been achieved, and over 12 million cases had been cured by 2002. Thus, the WHO Leprosy Programme set a target for the elimination of leprosy (less than 1 case per 10,000) by the year 2000. With the failure to achieve this goal, more recently, WHO formed a Global Alliance for the Elimination of Leprosy (GAEL) with the aim of reaching the elimination target (less than 1 case per 10,000) in all countries by 2005. However, to date, there is no clear evidence of an impact of introduction of MDT on the rate of detection of new cases. While global prevalence has dropped from the millions in the 1970s to less than 650,000 cases in 2002, new case detection has remained steady over the years at over 700,000 per annum. Approaches to address
this problem are impeded by a lack of fundamental knowledge about the epidemiology of leprosy, the sources of infection, its precise mode of transmission, and the importance of contact patterns.

The “Initiative for Diagnostic and Epidemiological Assays for Leprosy” (IDEAL) resulted from two workshops under the auspices of the TDR program of WHO. The first workshop, held in Geneva in November 2002, identified two fields of leprosy research in which advances are needed in order to eliminate leprosy. These are:

1) Nerve damage and 2) Early diagnosis and transmission.

In October 2003 a second workshop was organized in Amsterdam, in which the research needs in the field of early diagnosis and transmission were further identified and made explicit in a proposal for a comprehensive leprosy research program. This research program aims at the application of new developments in the fields of molecular typing of \( M. leprae \) and specific antigen/epitope definition to field studies towards better understanding of the epidemiology and transmission of leprosy, and the improved diagnosis of leprosy infection. The three main areas of research in this program are:

- Assays for molecular epidemiology
- Immunology-based diagnostic assays
- Field studies related to transmission and diagnosis

An Interim Steering Committee (Drs. Brennan, Dockrell, Engers, Klatser, Oskam, Richardus) was appointed to coordinate efforts to obtain funding and to invite partners (both research institutes and field programs) to join the consortium. The partners and sites chosen all have a proven track record in leprosy research, providing access to sufficient leprosy patients and their contacts within a functional leprosy control programme, with well-equipped laboratories and/or with experience in capacity building and technology transfer. The specific aims of the IDEAL research program are to:

1. Identify and develop \( M. leprae \)-specific proteins and peptides suitable for use in T cell assays, to enable specific immune responses to be identified in paucibacillary leprosy patients or contacts.
2. Dissect biomarkers identifying protective and non-protective immune responses in groups of leprosy contacts, that could be used to develop simple assays to identify infected subjects without protective immunity in leprosy-endemic countries.
3. Identify and assess the full range of polymorphisms at short tandem repeat (STR) and single nucleotides in the \( M. leprae \) genome with sufficient genetic variability to define sources of infection, transmission patterns and distinguish between new and reactivated cases of clinical leprosy.
4. Apply these new tools in field settings with different population characteristics and levels of endemicity.
5. Form a global platform for research groups so that research can be implemented in a coordinated manner, thus speeding up the quest for solutions to the issues of leprosy.

The research developments underlying these aspirations will be explained as also will the convening of the first meeting of IDEAL partners in Addis Ababa, Ethiopia, October 25–27, 2004 will be described.

—Patrick J. Brennan

\textit{Colorado State University}

\textbf{Regulation by clofazimine of cytokine production in \( M. leprae \)-infected macrophages}

Anti-mycobacterial drug, clofazimine or B663, has been used for the treatment of leprosy and some of the mycobacterial infections for the purpose of killing of the causative bacilli, moreover, the drug is reported to be useful in several immunologically mediated skin disorders. The mechanism of action by clofazimine is still unclear, although several studies have suggested its modulatory effects on immune response. In leprosy, clofazimine is also used for the suppression of leprosy reaction. To investigate the mechanism of immunomodulation by
clofazimine, the macrophage, one of the immune cells which play a very important role in leprosy as a host cell of \textit{M. leprae}, was studied on the basis of the effect of the drug on cytokine production in response to \textit{M. leprae}. By \textit{in vitro} study it was found that B663 enhanced TNF production in \textit{M. leprae}-stimulated mouse macrophages, moreover, the drug suppressed IL-10 and PGE2 production in the cells. The suppressive effect on IL-10 production could be due to the suppression of PGE2 production, since PGE2 was required to induce IL-10 by elevation of intracellular cAMP level through the stimulation of adenylate cyclase. PGE2 is a well-known inflammatory factor, therefore, anti-inflammatory activity of the drug could be due to the suppressive effect on PGE2 production. TNF is known as the co-activator of macrophages with IFN gamma, suggesting that B663 could enhance antibacterial activity of the host through the enhancement of TNF.

—Yasuo Fukutomi*, Fumihiko Takeshita**, Masanori Matsuoka* and Masahiko Makino*

\*Leprosy Research Center, National Institute of Infectious Diseases, Tokyo, and
\**Yokohama City University School of Medicine

**Nerve damage by bacteria causing Buruli ulcer—ultrastructure of mouse inoculated with \textit{Mycobacterium ulcerans}**

Buruli ulcer is an intractable skin disease caused by \textit{Mycobacterium ulcerans}. It is observed in tropical area such as Africa and Australia. Large, necrotizing, relatively painless, deep skin ulcers are formed mainly in the extremities. Because of chronic course and occasional complication of severe deformities, socioeconomic handicap is a great problem.

Recent study demonstrated that Phenolic Glycolipid-I (PGL-I), a \textit{Mycobacterium leprae}-specific membranous antigen responsible for Schwann cell invasion, is present in Buruli ulcer. Thus, we hypothesized that not only \textit{M. leprae} but also \textit{M. ulcerans} may invade peripheral nerve.

**MATERIALS AND METHODS**

Bacterial suspension of \textit{M. ulcerans} colony 97-107 cultured at 32°C in 7H9 culture medium (CFU = 1.3 × 10^9/ml, 25μl) was inoculated into the bilateral footpads of female BALB/c mice. Local swelling and redness were observed at day 33 after the inoculation, and sequential histopathological examination was performed since then.

Perfusion fixation by 10% formalin was done, and hind limbs were histopathologically examined by HE, acid-fast staining and immunohistochemistry using anti-PGL-I antibody. Also in selected cases, perfusion fixation by 2% glutaraldehyde was done, and hind limbs embedded in Epon, cut into 1μm were examined. When nerve damage is observed, electron microscopic examination was performed.

**RESULTS**

\textit{Day 33 after the inoculation of \textit{M. ulcerans}:} Dermal erosion and extensive edema of subcutaneous tissue were associated with infiltration of small number of neutrophils and monocyte. Granuloma formation was absent. Small clusters of long acid-fast bacilli were noted mainly in the stroma and in the cytoplasm of monocytes (Fig. 1). Peripheral nerves were well preserved even in the edematous lesion.

\textit{Day 55 after the inoculation of \textit{M. ulcerans}:} Remarkable deep skin ulcer and extensive subcutaneous edema were observed. Large number of acid-fast bacilli formed clusters in the edematous stroma. Many nerve bundles were well preserved, but some showed vacuolar change of Schwann cells (Fig. 2), and others were invaded by numerous acid-fast bacilli with massive nerve damage. Ultrastructurally, the bacilli were mainly in the endoneurium, and Schwann cells were spare (Fig. 3). PGL-I immunohistochemistry was negative.

**DISCUSSION**

Among the mycobacterial species, only \textit{M. leprae} is known to show neurotropism and causes nerve damage. In the previous studies, mild degenerative change with thickening of Schwann cell basal lamina and vacuolar change of axons were reported
{Mwanatambwe, 2000}, but direct nerve invasion by the acid-fast bacilli has not been found. Our study first demonstrated that nerve bundles are damaged by numerous *M. ulcerans*. This finding raises a new possibility of pathogenesis of “painlessness” of Buruli ulcer.

—Masamichi Goto\textsuperscript{1}, Hajime Saito\textsuperscript{2}, Kazue Nakanaga\textsuperscript{1}, Norihisa Ishii\textsuperscript{3}, Suguru Yonezawa\textsuperscript{1}

\textsuperscript{1}Kagoshima University, \textsuperscript{2}Hiroshima Environment and Health Association, \textsuperscript{3}National Institute of Infectious Diseases Leprosy Research Center

### Chemotherapy and Drug Resistance in Leprosy

The chemotherapy of leprosy, which is caused by *Mycobacterium leprae*, was launched in 1943 and dapsone was introduced as standard chemotherapy for leprosy in the 1950s. Between the 1960s and 1970s, other anti-leprosy agents such as clofazimine and rifampin were introduced due to the emergence of dapsone resistance resulted from long-term monotherapy with dapsone. The first dapsone resistant case was proved by mouse foot-pad method in 1964. To conquer the increasingly worldwide spread of dapsone resistance and control leprosy, the World Health Organization recommended multidrug therapy in 1981. Regimens included dapsone, rifampin and clofazimine in different doses and durations for multibacillary case (MB) and paucibacillary case (PB). Recently, ofloxacin and minocycline have been added for treating single lesion paucibacillary case (SPB).

Dapsone targets dihydropteroate synthase (DHPS) encoded by the *folP* and inhibits folic acid biosynthesis by acting as a competitive inhibitor of *p*-aminobenzoic acid (PABA). The target for rifampin is beta subunit of the RNA polymerase, encoded by the *ropB*, and transcription is inhibited. The mechanisms of antimicrobial activity for clofazimine has not been fully elucidated, however, the drug appears preference to bind to GC-rich sequences of mycobacteria. Similarly, the mechanism of bactericidal activity of minocycline against *M. leprae* is unknown, but thought to inhibit protein synthesis by blocking the binding of aminoacyl transfer RNA to the messenger RNA. Ofloxacin, one of new quinolones, it is likely to inhibit DNA replication by binding to A-subunits (GyrA) of DNA gyrase, a type II topoisomerase.

In spite of discovery of the genetic background for understanding of drug resistance in dapsone, rifampin and ofloxacin, only a limited number of mutations responsible for resistance have been reported in *M. leprae* to date since drug susceptibility of *M. leprae* to anti-leprosy drug has been tested by mouse foot-pad method. Almost all mutations in relevant genes conferring resistance to each drug were point mutations. Responsible point mutations, at 513 (1 case), 516 (1 case), 526 (3 cases), 531 (24 cases) and 533 (1 case) in the *rpoB*, were observed from 30 isolates and a 6-bp insertion was shown between 514 and 515 in one isolate. No mutation was detected in rifmapin resistance determining region of 65 wild type isolates. Meanwhile, mutations at 53 (8 cases) and 55 (10 cases) in the *folP* were detected in dapsone resistant isolates and 12 susceptible isolates showed no mutations in the gene. Three isolates resistant to ofloxacin harbored mutation at 91 in the *gyrA*.

The prevalence of drug resistance deduced by the mutation detection among re-
lapsed, intractable and new cases will be discussed. A method for rapid detection of resistant isolates and the significance of relapse by the persistence of susceptible bacilli will be also considered.

—Masanori Matsuoka

National Institute of Infectious Diseases, Leprosy Research Center

__________________________

Loop-Mediated Isothermal Amplification of the dnaA sequence for rapid detection of Mycobacterium leprae

For the establishment of differential diagnosis of mycobacterial species, a part of the nucleotide sequences of the mycobacterial DnaA protein gene was determined by PCR based sequencing. Clinically relevant 27 mycobacterial species and 46 clinical isolates of Mycobacterium avium, M. intracellulare and M. kansasii were analyzed. Although dnaA partial sequences of M. tuberculosis complex were identical to each other, all of the nontuberculous mycobacterial (NTM) laboratory strains and clinical isolates tested, were easily identified as the respective species. The partial dnaA sequence similarity between M. avium and M. intracellulare was 78.3%, and that of M. kansasii and non-pathogenic M. gastri was 83.6%. Rapidly growing groups of mycobacteria were clearly separated from other species in unrooted phylogenetic tree. Based on the amplified DNA sequences, species specific-primers were successfully designed for the target mycobacterium species, M. avium, M. intracellulare, M. kansasii, and M. gastri. These results demonstrate that the variable sequence in DnaA coding gene were species-specific and were potent for the development of accurate and rapid diagnosis of Mycobacterium species. To develop rapid and simple identification method, we used loop-mediated isothermal amplification (LAMP) for detection of Mycobacterium leprae, M. kansasii and M. gastri. LAMP method is a novel nucleic acid amplification method in which reagent reacts under isothermal conditions with high specificity, efficiency and rapidity. The whole procedure was quite simple, starting with mixing of reagents in a single tube, followed by an isothermal reaction during the reaction mixture is held at 63°C. The resulting amplicons are load to agarose gel electrophoresis. The only equipment needed for the amplification reaction is a heat block that furnishes a constant temperature. Species-specific primers for M. leprae were designed by targeting the dnaA gene and specificity were validated with 27 mycobacteria species and 8 clinical isolates of M. leprae. The assay had a detection limit 5pg of purified DNA with 60 min. incubation time. The sensitivity and reaction time for LAMP methods to detect of M. kansasii and M. gastri purified DNA were 10 pg., 30 min. and 1 ng, 60 min., respectively. The results demonstrate the variable sequence in dnaA gene was species-specific. Application on LAMP method was potent for the rapid diagnosis method of mycobacterial species and especially useful in development country.

—Tetsu Mukai, Yuji Miyamoto, Masanori Matsuoka, Toshio Yamazaki, Masahiko Makino

Leprosy Research Center, National Institute of Infectious Diseases, Japan.

__________________________

Polymorphism on the 5′ Flanking Region of IL12R2 Affects Establishment of Clinical Type of Leprosy.

The intensity of cell-mediated immune (CMI) responses in mycobacterial infection, determines individual differences in susceptibility to the diseases. These differences might be clarified from the viewpoint of T cell responsiveness against IL-12 in patients with leprosy since the disease shows the wide clinical spectrum due to the effect of their inherited factors. Leprosy, a chronic disease caused by the infection of Mycobacterium leprae (M. leprae), shows a wide spectrum of clinical features. Tuberculoid type of leprosy (T-lep) patients show high level of CMI responses against M. leprae, which results in the resistance to infection, whereas lepromatous type of leprosy (L-lep) patients show poor CMI responses (instead, rich antibody responses) against the pathogen which results
in the progressive form of the disease. Recently, it was reported that the IL-12Rβ2 was more highly expressed in tuberculoid lesions compared with lepromatous lesions, whereas IL-12Rβ1 expression was similar in both lesions. Then, we analyzed the polymorphisms on the 5′ flanking region of IL12RB2 to determine possible immunogenetical factors that affect CMI responses, by employing leprosy as model.

The polymorphisms were examined by using direct sequencing technique to compare the allele frequencies between 129 L-lep patients and 46 T-lep patients. Several SNPs, including −1035A>G, −1023A>G, −650delG and −465A>G SNPs, were detected on the 5′ flanking region of IL12RB2. Frequency of haplotype 1 (−1035A, −1023A, −650G, −464A), which exhibited the highest frequency in the general Japanese population, was significantly lower in L-lep patients as compared with findings in T-lep patients and healthy controls. Reporter gene assays using Jurkat T cells revealed that all haplotypes carrying one or more SNPs exhibited lower transcriptional activity as compared with haplotype 1. Moreover, it was also elucidated that activated T cells derived from the donors carrying haplotype 1 showed higher expression of IL-12Rβ2 mRNA in the presence of IL-12 by employing real-time PCR method.

These results suggest that SNPs on the 5′ flanking region of IL12RB2 affect the expression level of IL-12Rβ2 molecules, which may be implicated in individual differences in CMI responsiveness against mycobacterial antigens, thereby leading to the lepromatous and tuberculoid types of leprosy.

—Hideki Ohyama1, Koretsugu Ogata2, Kazu Takeuchi3, Masako Namisato4, Yasuo Fukutomi5, Motoharu Suzuki6, Yasushi Uemura6, Tohru Tsujimura1, Sho Matsushita6

1Hyogo College of Medicine, 2SHIMADZU Cooperation, 3Okayama University, 4National Kuryu Rakusen-En Sanatorium, 5National Institute of Infectious Disease, 6Saitama Medical School

Genotypic variation of M. leprae within a high endemic community.

Mycobacterium leprae is an obligate intracellular pathogen that is widely distributed around the globe. There are no recognized patho-vars or sub-types and the bacterium exhibits little genetic diversity. The only documented highly variable sequences are associated with variable number tandem repeat (VNTR) sequences distributed throughout the genome. We recently showed that VNTR polymorphisms could be used effectively to discriminate geographically diverse M. leprae strains used in the laboratory. Similar polymorphisms have been used with other bacteria to suggest phylogenetic relationships among worldwide isolates and to examine the dissemination dynamics of disease agents in populations. However, the sensitivity, specificity and predicative value of VNTR polymorphisms for describing these relationships among M. leprae are not yet known. To better understand these relationships we examined the utility for VNTR genotyping to discriminate M. leprae strain types in high endemic communities.

Using a battery of 7 VNTR loci we examined the genetic diversity of M. leprae strains recovered from 58 unrelated leprosy cases presenting in Karigiri, India over a ten year period. The alleles for microsatellites (GAA (21), GTA (9), AT (17), and TA (18)) were determined by direct sequencing of PCR products using forward and reverse primers. The alleles for minisatellites (12-5, 21-3, and 27-5) were determined by electrophoresis of fluorescently labeled PCR products in agarose gels. The various VNTR exhibited diversity in alleles ranging from 0.3 to 0.9 in patient samples and successfully discriminated a total of 58 different VNTR genotypes among the 58 Karigiri cases tested. No epidemiologically significant relationships were discerned. To better understand the likelihood of chance influencing the remarkable discrimination observed, we used the same battery of VNTR to examine M. leprae from multiple tissue samples recovered from naturally infected wild nine-banded armadillos.

M. leprae is intensely transmitted within armadillo communities in Louisiana. Inci-
Disease density rates exceeding 3.5 cases/1000 animal-days have been measured and the disease may approximate an outbreak situation for *M. leprae* among armadillos. We recovered a total of 8 naturally infected wild armadillos from 2 Louisiana research sites during a 3 month period in 2004. *M. leprae* were harvested and the genotype of the bacilli determined from each of 8 different tissue samples for each animal. VNTR alleles showed markedly less diversity among armadillos than among the Karigiri patient samples. GAA remained the most highly diverse locus with other loci exhibiting greater stability. Analysis of *M. leprae* from the different tissues of each animal showed nearly identical VNTR genotypes and consistency in genotypes discerned for animals from each location.

VNTR genotyping can have high discriminatory power for differentiating *M. leprae* with high specificity. Additional studies addressing the appropriate mixture of alleles to be used and their combined sensitivity, specificity and predicative value are warranted.

—Richard W. Truman, James E. Adams, Gigi Ebenezer,* and Thomas P. Gillis

*National Hansen's Disease Programs, Baton Rouge, LA USA, and*  
*Schieffelin Leprosy Research and Training Center, Karigiri, Vellore, India*
ACKNOWLEDGMENT

The Board of Directors of the INTERNATIONAL JOURNAL OF LEPROSY gratefully acknowledges the financial assistance from special grantors and sustaining members which, with the special donations of certain members, has made possible the continuation of publication of the JOURNAL directly by the International Leprosy Association. Without this assistance the official organ of the ILS, so essential to leprosy workers everywhere, could not be published.

SPECIAL GRANTORS

*Aide aux Lepreux Emmaus-Suisse, Spitalgasse, CH-3011 Berne, Switzerland.

*American Leprosy Missions, One ALM Way, Greenville, South Carolina 29601, U.S.A.

*Amici dei Lebbrosi, Foundazione Italiana Raoul Follereau, Via Borselli 4, 40135 Bologna, Italy.

Damien-Dutton Society, 616 Bedford Avenue, Bellmore, New York 11710, U.S.A.

*Damien Foundation (DF/APD), 16 Rue Stevin, B-1040 Bruxelles, Belgium.

*Deutsches Aussatzigen-Hilfswerk e. V., Postfach 9062, D-97090 Würzburg 11, Germany.

*Le Secours aux Lépreux (Canada), 1275 Rue Hodge Bureau 12, Montreal H4N 3H4, Canada

*Netherlands Leprosy Relief, Wibautstraat 137K, 1097 DN Amsterdam, The Netherlands.

*Pacific Leprosy Foundation, 115 Sherborne Street, Bag 4730, Christchurch, New Zealand.