BIOLOGIA MOLECULAR/GENÉTICA

Lavania M, Katoch K, Singh H, Das R, Gupta AK, Sharma R, Chauhan DS, Sharma VD, Sachan P, Sachan S, Katoch VM. Predominance of three copies of tandem repeats in rpoT gene of Mycobacterium leprae from Northern India. Infect Genet Evol 2007; 7(5): 627-31.

This study has been carried out to get understanding of the origin among the strains of Mycobacterium leprae in patients from Northern India by using number of tandem repeats in rpoT gene as marker. Biopsies were collected from hundred leprosy cases (paucibacillary (PB) as well as multibacillary (MB)) across the spectrum from patients attending clinic at JALMA or diagnosed in Field Unit at Ghatampur (Kanpur). These biopsies were homogenized and DNA was extracted by a physiochemical procedure. rpoT region was amplified by using the primers and conditions earlier published. Among 100 strains from Northern Indian patients, 89% exhibited the presence of three copies of the 6bp tandem repeat in the rpoT gene, while 11% contained four copies. These profiles along with other genotyping data may help in studyi ng the historical spread of leprosy by strains of M. leprae disseminated by various human races that migrated to Northern India from other places of Asian continent.

Gupta N, Shankernarayan NP, Dharmalingam K. Serum proteome of leprosy patients undergoing erythema nodosum leprosum reaction: regulation of expression of the isoforms of haptoglobin. J Proteome Res 2007; 6(9): 3669-79.

Validated proteome profile allows better understanding of disease progression, subtype classification, susceptibility patterns, and disease prognosis. Leprosy is a spectral disease, with clinically, histologically, immunologically, and bacteriologically distinguishable subtypes. In addition, a significant fraction of patients undergo immune mediated reactions even after multidrug therapy (MDT). Erythema nodosum leprosum (ENL) is an immune complex mediated reactional condition in leprosy, characterized by a systemic inflammatory condition afflicting borderline lepromatous (BL) and lepromatous leprosy patients (LL). In this study, we have analyzed serum proteome of leprosy patients undergoing ENL reactions and compared it with that of healthy noncontact controls. Depletion of albumin and immunoglobulin G (IgG) was optimized using Aurum serum protein mini kit (Bio-Rad), and then two-dimensional gel electrophoresis (2-DE) of these serum samples was performed. Differentially expressed proteins were identified by MALDI-TOF and MALDI-TOF MS/MS mass spectrometry. Significant increase in one of the isoforms of alpha2 chain of haptoglobin was observed in ENL condition. In addition, haptoglobin phenotype was determined for healthy controls and leprosy patients. Hp 0-0 phenotype was detected in 21.4% of the ENL patients undergoing treatment, which on follow up examination showed typable phenotype, thus showing a condition of acquired anhaptoglobinemia. Since ENL still remains a threat to leprosy disease management, the above findings may provide new insights in understanding the development and progression of this inflammatory condition.

Ranque B, Alter A, Mira M, Thuc NV, Thai VH, Huong NT, Ba NN, Khoa PX, Schurr E, Abel L, Alcaïs A. Genomewide linkage analysis of the granulomatous mitsuda reaction implicates chromosomal regions 2q35 and 17q21. J Infect Dis 2007; 196(8): 1248-52.

The Mitsuda reaction, a delayed granulomatous skin reaction elicited by the intradermal injection of heat-killed Mycobacterium leprae, is an in vivo test reflecting the ability to generate an immune granuloma after sensitization by diverse mycobacterial infections. Accumulating evidence for the genetic control of the Mitsuda reaction has been reported. We performed a genomewide linkage scan for the quantitative Mitsuda reaction in 19 large families from Vietnam with a history of leprosy (114 offspring). Suggestive linkage was found at chromosomal regions 2q35 (P = 9 x 10(-4) at the SLC11A1 locus) and 17q21-25 (P = 8 x 10(-4)). Interestingly, these 2 regions have been previously linked to mycobacterial infection and other granulomatous diseases.

Messias-Reason IJ, Boldt AB, Moraes Braga AC, Von Rosen Seeling Stahlke E, Dornelles L, Pereira-Ferrari L, Kremsner PG, Kun JF. The association between mannan-binding lectin gene polymorphism and clinical leprosy: new insight into an old paradigm. J Infect Dis 2007; 196(9): 1379-85.

BACKGROUND: Mannan-binding lectin (MBL), a soluble protein of innate immunity, is known to play a role in pathogen recognition and clearance. For more than a decade, it has been proposed that MBL deficiency may be protective against intracellular pathogens, such as Mycobacterium leprae. METHODS: The polymorphisms at the promoter and exon 1 regions of the MBL2 gene were assessed by polymerase chain reaction and sequencing performed on 264 patients with leprosy and 214 matched healthy control subjects from southern Brazil. RESULTS. The distribution of MBL2-gene polymorphisms in patients was significantly different from that in controls, with a decreased frequency of haplotypes/genotypes associated with low expression of circulating MBL in lepromatous patients when compared with tuberculoid patients (odds ratio [OR] for haplotypes, 0.56 [95% confidence interval {CI}, 0.33-0.93] [P=.020]; OR for genotypes, 0.31 [95% CI, 0.13-0.71] [P=.004]). The LYPA haplotype was associated with susceptibility to leprosy per se (OR, 2.25 [95% CI, 1.31-3.88] [P=.003]) and to progression to the lepromatous (OR, 2.2 [95% CI, 1.21-4.05] [P=.008]) and borderline (OR, 2.98 [95% CI, 1.29-6.87] [P=.008]) forms of the disease. CONCLUSIONS: These results suggest that MBL2-gene polymorphisms play a role in susceptibility to leprosy per se and in the clinical progression of the disease.

Katoch VM, Lavania M, Chauhan DS, Sharma R, Hirawati, Katoch K. Recent advances in molecular biology of leprosy. Indian J Lepr 2007; 79(2-3): 151-66.

The last three decades have witnessed rapid progress in understanding the molecular biology of Mycobacterium leprae. Following the availability of complete genome sequence of leprosy bacillus in 2001, things have drastically changed. With the information about genetic structure, several techniques have been developed for diagnosis, molecular epidemiology and also detection of drug resistance. With the decline in the prevalence of leprosy globally, there has been some reduction in interest in the molecular methods for diagnosis, yet molecular techniques for studying the transmission dynamics and detection of drug resistance continue to be relevant. Knowledge about complete genome sequence has made it possible to undertake studies that can improve our understanding of the structure and function of this enigmatic organism. Newer information emerging about biology of M. leprae would provide insight into mechanisms of its survival and persistence in host and is likely to lead to better diagnostics and also therapeutics for mycobacterial infections in general.

Pieroni F, Stracieri, Moraes DA, Paton EJ, Saggioro FP, Barros GM, Barros JC, Oliveira MC, Coutinho MA, Castro NS, Vigoritto AC, Trabasso P, Souza CA, Souza MP, Mauad MA, Colturato VA, Simoes BP, Foss NP, Voltarelli JC. Six cases of leprosy associated with allogeneic hematopoietic SCT. Bone Marrow Transplant 2007; 40(9): 859-63.

We report here the first six cases of leprosy associated with HLA-identical allogeneic SCT in different phases and with different findings and outcomes. Skin and peripheral nerves may be sites of leprosy associated with SCT, stressing the importance of differential diagnosis between leprosy and GVHD or drug reactions. Clinical manifestations of leprosy before or after transplantation did not influence the outcome of SCT in our cases.

CLÍNICA

Chaudhry IA, Shamsi FA, Elzaridi E, Awad A, Al-Fraikh H, Al-Amry M, Al-Dhibi H, Riley FC. Initial diagnosis of leprosy in patients treated by an ophthalmologist and confirmation by conventional analysis and polymerase chain reaction. Ophthalmology 2007; 114(10): 1904-11.

PURPOSE: To report the initial diagnosis of leprosy in patients seeking treatment from an ophthalmologist in a tertiary eye care center, its confirmation by histopathologic and polymerase chain reaction analysis, and review of literature. DESIGN: Noncomparative retrospective case series. PARTICIPANTS: Patients with no known history of leprosy who were selected based on either a clinical suspicion or a histopathologic diagnosis and were found to have ocular or periocular lesions suggestive of leprosy. METHODS: Review of clinical records of patients with no known history of leprosy seen by an ophthalmologist at a tertiary eye care referral center. MAIN OUTCOME MEASURES: Patient demographics, presenting symptoms and signs, diagnostic studies, complications, and treatment. RESULTS: Among the 6 patients (5 women and 1 man; average age, 55 years), only 2 were found to have leprosy from clinical examination alone. Histopathologic characteristics or demonstration of acid-fast bacilli, suggestive of leprosy, were found in 5 patients. Definite confirmation of leprosy was made by polymerase chain reaction performed on formalin-fixed, paraffin-embedded tissues of 4 patients having suspected leprosy based on clinicohistopathologic examination results. CONCLUSIONS: The diagnosis of leprosy relies on the clinical symptom complex, epidemiologic factors, and demonstration of acid-fast bacilli in the tissue sample. Considering that the global leprosy population is 12 million, a patient with leprosy may be found anywhere. The ophthalmologist may be the first one to encounter such patient, in which case suspicion and detection of ocular findings may lead to early treatment of the infection. Polymerase chain reaction may be a new tool in the definite diagnosis of leprosy when suspicion of the diseas es is raised by clinicohistopathologic studies.

Pereyra SB, Danielo CA, Ponssa GJ, Consigli JE, Papa MB, Ghirardi G. Wade's histoid leprosy: three clinical presentations. Int J Dermatol 2007, 46(9): 944-6.

Wade's histoid leprosy is a rare form of multibacillary leprosy with distinctive characteristics which were first described in patients treated with a short course of sulfones. Wade's histoid leprosy may occur as a relapse, in the setting of sulfone resistance, or may present de novo. We report the clinical, histologic, immunologic, and bacteriologic features of three adult male patients with this rare variant of lepromatous leprosy as the initial presentation of the disease, observed in the Dermatology Department of Hospital Córdoba, Córdoba, Argentina between 1999 and 2003. Two of the three patients were from an endemic leprosy area. All patients presented with a number of erythematous to brownish gray, firm but elastic nodules involving mainly the extremities, which responded to treatment for multibacillary leprosy without reactional episodes.

Ribeiro FB, Pereira FA, Muller E, Foss NT, Paula FJ. Evaluation of bone and mineral metabolism in patients recently diagnosed with leprosy. Am J Med Sci 2007; 334(5): 322-6.

This study was conducted to evaluate patients recently diagnosed with the tuberculoid and lepromatous forms of leprosy for bone mass, bone remodeling, and hormones related to mineral control. Eleven normal control individuals (CG) and 12 patients with leprosy (LG) matched for physical characteristics were submitted to evaluation of bone mass density (BMD) and to the determination of serum levels of PTH, 25-hydroxyvitamin D [25(OH)D], testosterone, LH, FSH, osteocalcin (OC), and urinary levels of deoxypyridinoline (DPD). The T score of lumbar spine and total radius (mean +/- SD) were significantly lower in leprosy patients (L1-L4: CG = -0.7 +/- 1.5 vs LG = -1.8 +/- 1.0 SD, $P < 0.04$, and total radius: CG = -1.43 +/- 0.6 vs LG = -2.1 +/- 0.8 SD, $P < 0.02$), whereas no significant differences were observed i n

total hip or femoral neck T score. However, at all sites, the rate of low bone mass (T score < -1.0) was higher in LG (femoral neck: CG = 18% vs LG = 50%, total hip: CG = 27% vs LG = 42%). There was a significant difference in albumin and PTH levels between groups but not in serum 25(OH)D and OC levels or urinary DPD levels. The present results indicate that bone mass loss is an early event in leprosy patients and frequently is already present at diagnosis. Its etiopathogenesis is multifactorial, and further studies are needed to determine the most efficient way to prevent fractures in this condition. The data obtained in the present study need confirmation by the evaluation of a larger sample.

DIAGNÓSTICO / CLÍNICO

Ishii N, Suzuki K, Takezaki S, Nagaoka Y. [Summary of questionnaires on slit skin smear test in clinics, hospitals, and university hospitals]. Nihon Hansenbyo Gakkai Zasshi 2007; 76(3): 227-32.

We have performed a questionnaire to survey the present conditions of the slit skin smear test, a method to diagnose leprosy. The answer was obtained from 40 (93.0%) out of 43 clinics, department of dermatology of university and other hospitals and leprosy sanatoriums. Slit skin smear test was carried out in most institutions. However, when inspection frequency was low, a laboratory technician performed Ziehl-Neelsen staining or its variation using a normal slide glass. A few institutions carried out fluorescence staining. Both physician and technician examined the slides in most cases, however, in more than half of the leprosy sanatoriums, technician was the only person examined. Bacterial index was evaluated in most institutions, while only for the presence of bacteria was examined 5 institutions. Sl it skin smear test is simple and easy, but accuracy is different with skills, glass slides to be used and methods for staining and inspection. Supply of the glass slide with a marker, the spread of staining methods, technical improvement of an inspection are demanded in future.

EPIDEMIOLOGIA / CONTROLE

Souza Dias MC, Dias GH, Nobre ML. The use of Geographical Information System (GIS) to improve active leprosy case finding campaigns in the municipality of Mossoró, Rio Grande do Norte State, Brazil. Lepr Rev 2007; 78(3): 261-9.

There is a high incidence of leprosy in the municipality of Mossor6, Rio Grande do Norte state, where the detection coefficient has risen from 2.78/10,000 population in 1998 to 5.14 in 2004. While cases have been registered throughout the urban area, the disease is concentrated in select neighbourhoods. This study was undertaken using Geographical Information System (GIS) with the objective of defining low-cost, effective strategies to control leprosy. The land registry map of the city, Ikonos satellite images and the SINAN (National Morbidity Notification Information System) database were used as the cartographical basis for the study. The sample for the leprosy mapping was drawn from the 358 new cases of the disease diagnosed in the municipality between 1998 and 2002. The houses of 281 patients were located (78.5% of the total) and their addresses geo-referenced using a GPS handheld device. Subsequently, geographical analysis was carried out using ArcView 9.0 software showing predominant concentration of cases in the neighbourhoods of Barrocas, Santo Antônio, Bom Jardim and Paredoes. This mapping served as the basis for four active case finding campaigns conducted in the most highly concentrated areas between March and September of 2005. Campaigns guided by spatial analysis led to the diagnosis of 104 new cases of the disease (50% of the total number of new cases detected in the municipality in 2005). The use of GIS in leprosy diagnosis has shown to be extremely effective, providing a clear visual understanding of the distribution of the disease in the municipality, which results in targeted interventions and important cost reductions in leprosy control activities.

IMUNOLOGIA

Hagge DA, Marks VT, Ray NA, Dietrich MA, Kearney MT, Scollard DM, Krahenbuhl JL, Adams LB. Emergence of an effective adaptive cell mediated immune response to Mycobacterium leprae is not impaired in reactive oxygen intermediate-deficient mice. FEMS Immunol Med Microbiol 2007; 51(1): 92-101.

Cytokine-activated macrophages (MPhi) employ reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) to combat pathogens. The requirement for ROI for an effective host response to experimental leprosy using mice which have a disruption in the 91-kD subunit of the NAPDH oxidase cytochrome b (phox91-/-) was examined. Mycobacterium leprae multiplication in phox91-/- foot pads (FP) was elevated early in infection but subsequently arrested similarly to control mice within a noninvasive granuloma. Using a modified lepromin test model, a similar cellular composition in the M. leprae-induced FP granuloma in both strains with lymphocyte infiltration consisting primarily of CD4+CD44(hi)CD62L(lo) effector cells was found. Of great interest was the disparity in the T cell population between t he granuloma and the draining lymph node which contained predominantly naïve CD4+CD44(lo) CD62L(hi) cells and was, therefore, not representative of the infection site. TH1 cytokines, chemokines and inducible nitric oxide synthase were comparably expressed in the FP of both strains. When infected in vitro, normal MPhi from B6 and phox91-/- mice supported bacterial viability, whereas IFNgamma-activated MPhi killed M. leprae in a RNI-dependent manner, emphasizing that ROI was dispensable. These data show that phox91-/- mice generate a strong adaptive immune response and control long-term infection with M. leprae.

Moura DF, Teles RM, Ribeiro-Carvalho MM, Teles RB, Santos IM, Ferreira H, Fulco TO, Nery JA, Sampaio EP, Sarno EM. Long-term culture of multibacillary leprosy macrophages isolated from skin lesions: a new model to study Mycobacterium leprae-human cell interaction. Br J Dermatol 2007; 157(2): 273-83.

BACKGROUND: Leprosy is characterized by a disease spectrum having two polar clinical forms dependent on the presence or not of cell-mediated immunity. In the tuberculoid forms, granuloma-activated macrophages kill Mycobacterium leprae in conjunction with a Th1 response while, in multibacillary (MB) lesions, M. leprae nonactivated macrophages infiltrate the nerves and internal organs together with a Th2 response. The functional properties and activation pathways of macrophages isolated from patients with MB leprosy remain only partially understood. OBJECTIVES: To establish an ex vivo methodology capable of evaluating the activation pathways, grade and fate of cultured macrophages isolated from MB lesions. METHODS: Skin biopsies from patients with borderline tuberculoid, borderline lepromatous and leproma tous leprosy (LL) were characterized by immunohistochemistry and transcriptional analysis. To isolate inflammatory cells, a portion of the samples was submitted to enzymatic digestion. These same cells, maintained in culture for a minimum 7-day period, were characterized morphologically and via flow cytometry at different culture time points. Cytokine [interferon (IFN)-gamma, tumour necrosis factor (TNF)-alpha and interleukin (IL)-10] mRNA levels were quantified by real-time polymerase chain reaction and protein secretion in the culture supernatants was measured by enzyme-linked immunosorbent assay and the nitric oxide levels by Griess reagent. RESULTS: RNA expression in tuberculoid and MB lesions showed the profile expected of characteristic Th1 and Th2 responses, respectively. The inflammatory cells in all biopsies were successfully isolated. Although the number of cells varied between biopsies, it was highest in LL biopsies. The frequency of isolated CD14+ and CD3+ cells measur ed by flow cytometry correlated with the percentages of macrophages and lymphocytes in the lesions. Throughout the culture period, CD68+ macrophages showed morphological changes. A progressive increase in cell number and reduction of infected cells were perceptible in the cultures. In contrast to the biopsies, TNF-alpha, IFN-gamma and IL-10 expression in the tuberculoid and MB leprosy cells in 24-h culture and the cytokine levels in the supernatants did not differ significantly. During the culture period, cytokine expression in the MB cells progressively declined, whereas, from days 1 to 7, nitrite levels progressively increased. After day 40, the remaining macrophages were able to ingest fluorescein isothiocyanate-labelled M. leprae. These data need to be confirmed. CONCLUSIONS: This study confirmed the feasibility of obtaining ex vivo macrophages from leprosy lesions and keeping them in long-term culture. This procedure may open new pathways to studying the interaction between M. leprae and human macrophages, which might, in turn, lead to the development of therapeutic tools capable of overcoming the specific anergy found in patients with MB leprosy.

Rodrigues LC, Kerr-Pontes LR, Frietas MV, Barreto ML. Long lasting BCG protection against leprosy. Vaccine 2007; 25(39-40): 6842-4.

BACKGROUND: BCG vaccine protects against leprosy. OBJECTIVES: Estimate BCG protection against leprosy by age by age. METHODS: A case control study with 226 cases of leprosy and 857 controls. BCG vaccination was ascertained via examination of BCG scars. Protection is presented for three age groups. RESULTS: BCG protection against leprosy was 86% (95% CI: 77-92%) in the age group 18-29; 54% (95% CI: -37% to 85%) in the age group 30-39 and 32% (95% CI: -3% to 56%) in those aged 40 or more. CONCLUSIONS: BCG efficacy against leprosy may well last for three decades and possibly even longer. BCG vaccination must have contributed to worldwide reduction in leprosy incidence.

Duthie MS, Reece ST, Lahiri R, Goto W, Raman VS, Kaplan J, Ireton GC, Bertholet S, Gillis TP, Krahenbuhl JL, Reed SG Antigen-specific cellular and humoral responses are induced by intradermal Mycobacterium leprae infection of the mouse ear. Source: Infect Immun 2007; 75(11): 5290-7.

Leprosy is caused by infection with Mycobacterium leprae. The immune response of leprosy patients can be highly diverse, ranging from strong cellular responses accompanied by an apparent deficit of M. leprae-specific antibodies to strong humoral responses with a deficit of cell-mediated responses. Leprosy takes many years to manifest, and this has precluded analyses of disease and immune response development in infected humans. In an attempt to better define development of the immune response during leprosy we have developed an M. leprae ear infection model. Intradermal inoculation of M. leprae into the ear supported not only infection but also the development of a chronic inflammatory response. The inflammatory response was localized, comprising a T-cell infiltration into the ear and congestion of cel ls in the draining lymph nodes. The development of local chronic inflammation was prevented by rifampin treatment. Importantly, and in contrast to subcutaneous M. leprae footpad infection, systemic M. leprae-specific gamma interferon and antibody responses were detected following intradermal ear infection. These results indicate the utility of intradermal ear infection for both induction and understanding of the immune response during M. leprae infection and the identification or testing of new leprosy treatments.

Iyer A, Hatta M, Usman R, Luiten S, Oskam L, Faber W, Geluk A, Das P. Serum levels of interferon-gamma, tumour necrosis factor-alpha, soluble interleukin-6R and soluble cell activation markers for monitoring response to treatment of leprosy reactions. Clin Exp Immunol 2007; 150(2): 210-6.

Identifying pathogen and host-related laboratory parameters are essential for the early diagnosis of leprosy reactions. The present study aimed to clarify the validity of measuring the profiles of serum cytokines [interleukin (IL)-4, IL-6, IL-10, interferon (IFN)-gamma and tumour necrosis factor (TNF)-alpha], the soluble IL-6 receptor (sIL-6R), soluble T cell (sCD27) and macrophage (neopterin) activation markers and Mycobacterium leprae-specific anti-PGL-I IgM antibodies in relation to the leprosy spectrum and reactions. Serum samples from 131 Indonesian leprosy patients (82 non-reactional leprosy patients and 49 reactional) and 112 healthy controls (HC) from the same endemic region were investigated. Forty-four (89.8%) of the reactional patients had erythema nodosum leprosum (ENL) while only five (10. 2%) had reversal reaction (RR). Follow-up serum samples after corticosteroid treatment were also obtained from 17 of the patients with ENL and one with RR. A wide variability in cytokine levels was observed in the patient groups. However, IFN-gamma and sIL-6R were elevated significantly in ENL compared to non-ENL patients. Levels of IFN-gamma, TNF-alpha and sIL-6R declined significantly upon corticosteroid treatment of ENL. Thus, although the present study suggests limited applicability of serial measurement of IFN-gamma, TNF-alpha and sIL-6R in monitoring treatment efficacy of ENL, reactions it recommends a search for a wider panel of more disease-specific markers in future studies.

Sekar B. Recent advances in immunodiagnosis of leprosy. Indian J Lepr 2007; 79(2-3): 85-106.

Although prevalence of leprosy is considerably reduced, the unabated emergence of about 300,000 cases worldwide indicates that the source of infection and transmission are not being addressed. Early diagnosis and treatment still remain the cornerstone of leprosy control. Many diagnostic issues hinder the correct and timely diagnosis and classification of leprosy. Delayed and missed diagnosis of infectious leprosy patients and the lack of tests to measure asymptomatic M. leprae infection in contacts also hamper the assessment of transmission of M. leprae infection. An important goal would be the development of improved diagnostic tools to diagnose difficult cases and to detect M. leprae infection before clinical manifestation. The search for

an ideal immunodiagnostic tool for leprosy had gone through various phases and development over the years, with inherent limitations in the sensitivity and specificity of the immunodiagnostic tests for leprosy. With improvement in technology many modifications of previously used PGL-1 assay in the form of rapid and less expensive techniques, such as dipstick, ELISA, ML flow test, have been introduced. Many new skin test antigens with potential for improving their efficiency, such as MLSA LAM, MLCwA and their fractionates, have been studied. After the completion of genome sequencing of M. leprae in 2000, many genes that were studied in M. tuberculosis and found potential for the immunodiagnosis of tuberculosis, such as CFP-10 and ESAT-6 proteins, have been investigated in M. leprae also. Genes that are unique to M. leprae with no homologous in M. tuberculosis have been explored for novel M. leprae-specific antigens. In order to overcome the problem of cross-reactivity, a number of workers have synthesized overlapping short peptides of differen t M. leprae recombinant proteins and studied their sequence divergence and attempted to identify M. leprae-specific B- and T-cell epitopes. This review makes an effort to present an overview of all these developments in the field of immunodiagnosis of leprosy.

Belgaumkar VA, Gokhale NR, Mahajan PM, Bharadwaj R, Pandit DP, Deshpande S. Circulating cytokine profiles in leprosy patients. Lepr Rev 2007; 78(3): 223-30.

BACKGROUND: Leprosy is a chronic infectious disease caused by Mycobacterium leprae which is an obligate intracellular pathogen. It is characterised by a broad spectrum of clinical forms dictated by the patient's immune response to the organism. The tuberculoid pole has good cell mediated immunity to M. leprae, with few lesions and bacilli while the lepromatous pole has poor immunity coupled with extensive involvement and greater bacillary load. METHODS: We studied serum levels of interferon gamma and interleukin 6 in 100 patients of untreated leprosy, compared them with 30 age and sex matched normal healthy controls and corelated them with different parts of the spectrum and reactional episodes. The purpose of this study was to delineate the role of cytokines and their clinical implications in the lep rosy spectrum and during reactional episodes. RESULTS: We observed that mean cytokine levels were significantly higher in the patient group as compared to the controls. In the non reactional patient group, pure neuritic leprosy patients showed highest levels of INFgamma which were directly proportional to the extent of nerve involvement. Lepromatous leprosy patients had the highest levels of IL6. Bacteriological index demonstrated a negative and positive corelation with INFgamma and IL 6 levels respectively. Type I and Type II reactional patients had higher levels of INFgamma and IL 6 respectively as compared to nonreactional patients. CONCLUSIONS: Our results suggest that pure neuritic leprosy and borderline tuberculoid patients in type I reaction are at greatest risk for nerve and tissue damage. Thus cytokines have the potential to play a significant role in classification, prognosis and treatment of leprosy.

Bhandarkar SS, Cohen C, Kuruvila M, Rea TH, Mackelfresh JB, Lee DJ, Modlin RL, Arbiser JL. Angiogenesis in cutaneous lesions of leprosy: implications for treatment. Arch Dermatol 2007; 143(12): 1527-9.

OBJECTIVE: To examine the potential role of angiogenesis in leprosy. DESIGN: Immunohistochemical analysis of leprosy lesions. SETTING: Department of Dermatology, Venereology, and Leprology, Kasturba Medical College; Division of Dermatology, University of California at Los Angeles; and Departments of Dermatology and Pathology, Emory University. PATIENTS: Thirty-two cutaneous lesions that represented the spectrum of leprosy were obtained from 32 patients. MAIN OUTCOME MEASURE: CD31 microvessel counts. RESULTS: The mean CD31 microvessel count in borderline tuberculoid, midborderline, and lepromatous leprosy lesions was significantly higher than in indeterminate leprosy lesions. CONCLUSIONS: Increased bacterial load is associated with increased angiogenesis. Angiogenesis inhibitors may be of benefit in the treatment of leprosy.

Silva EA, Iyer A, Ura S, Lauris JR, Naafs B, Das PK, Vilani-Moreno F. Utility of measuring serum levels of anti-PGL-I antibody, neopterin and C-reactive protein in monitoring leprosy patients during multi-drug treatment and reactions. Trop Med Int Health 2007; 12(12): 1450-8.

OBJECTIVE: To verify the validity of measuring the levels of Mycobacterium leprae-specific anti-phenolic glycolipid (PGL)-I antibody, neopterin, a product of activated macrophages, and C-reactive protein (CRP), an acute phase protein, in serial serum samples from patients for monitoring the leprosy spectrum and reactions during the course of multi-drug treatment (MDT). METHODS: Twenty-five untreated leprosy patients, 15 multi-bacillary (MB) and 10 paucibacillary (PB), participated. Eight patients developed reversal reaction and five developed erythema nodosum leprosum (ENL) during follow-up. The bacterial index (BI) in slit-skin smears was determined at diagnosis and blood samples collected by venipuncture at diagnosis and after 2, 4, 6 and 12 months of MDT. PGL-I antibody and neopterin were measured b y enzyme-linked immunosorbent assay, whereas the CRP levels were measured by the latex agglutination method. RESULTS: The levels of PGL-I antibodies and neopterin were higher in the sera of MB than PB patients, which correlated with the patients' BI. The serum levels of CRP did not differ significantly between the MB and PB patients. The serum levels of PGL-I and neopterin were no higher in reactional patients than non-reactional patients prone to such reactions. However, ENL patients had higher serum CRP levels than non-reactional MB patients. The serum PGL-I antibody levels declined significantly during MDT, in contrast to neopterin and CRP levels. CONCLUSION: Measuring the serum levels of PGL-I antibodies and neopterin appeared to be useful in distinguishing MB from PB patients, whereas monitoring the levels of PGL-I antibodies appeared to be useful in monitoring MB patients on MDT. Measuring serum CRP, although not useful in monitoring the patients, has limited significance in detecting ENL reactional patients.

Duthie MS, Goto W, Ireton GC, Reece ST, Cardoso LP, Martelli CM, Stefani MM, Nakatani M, Jesus RC, Netto EM, Balagon MV, Tan E, Gelber RH, Maeda Y, Makino M, Hoft D, Reed SG. Use of protein antigens for early serological diagnosis of leprosy. Clin Vaccine Immunol 2007; 14(11): 1400-8.

Leprosy is a chronic and debilitating human disease caused by infection with the Mycobacterium leprae bacillus. Despite the marked reduction in the number of registered worldwide leprosy cases as a result of the widespread use of multidrug therapy, the number of new cases detected each year remains relatively stable. This indicates that M. leprae is still being transmitted and that, without earlier diagnosis, M. leprae infection will continue to pose a health problem. Current diagnostic techniques, based on the appearance of clinical symptoms or of immunoglobulin M (IgM) antibodies that recognize the bacterial phenolic glycolipid I, are unable to reliably identify early-stage leprosy. In this study we examine the ability of IgG within leprosy patient sera to bind several M. leprae protein antigens. As expected, multibacillary leprosy patients provided stronger responses than paucibacillary leprosy patients. We demonstrate that the geographic locations of the patients can influence the antigens they recognize but that ML0405 and ML2331 are recognized by sera from diverse regions (the Philippines, coastal and central Brazil, and Japan). A fusion construct of these two proteins (designated leprosy IDRI diagnostic 1 [LID-1]) retained the diagnostic activity of the component antigens. Upon testing against a panel of prospective sera from individuals who developed leprosy, we determined that LID-1 was capable of diagnosing leprosy 6 to 8 months before the onset of clinical symptoms. A serological diagnostic test capable of identifying and allowing treatment of early-stage leprosy could reduce transmission, prevent functional disabilities and stigmatizing deformities, and facilitate leprosy eradication.

Chattree V, Khanna N, Rao DN. Alterations in T cell signal transduction by M. leprae antigens is associated with downregulation of second messengers PKC, calcium, calcineurin, MAPK and various transcription factors in leprosy patients. Mol Immunol 2007; 44(8): 2066-77.

Mycobacterium leprae, the causative agent of leprosy, challenges host defense mechanism by impairing the signal transduction of T cells which leads to downregulation of T cell proliferation, mainly as a consequence of interference with IL-2 production. In this study we sought to identify how soluble forms of M. leprae antigen(s) or particulate (liposome) delivery of the same antigens with two immunomodulators Murabutide and T cell peptide of Trat protein influence the transcription of IL-2 gene in anergic T cells of lepromatous patients. It was demonstrated that MLCwA/ManLAM stimulated cells of BL/LL patients showed defects in both jun-NH2-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK) activities there by resulting in decreased AP-1 activity. Additionally these cells showed reduc ed calcium levels, PKC activity and calcineurin (CN) activity. This led to impaired nuclear translocation of NFkappaB and NFAT in these patients. In contrast, when same M. leprae antigen(s) were incorporated with the two immunomodulators in liposomal form, increased transcription of IL-2 gene was observed especially in BL/LL patients which appears to be due to, at least in part, to increased expression of AP-1 Fos and Jun family members, NFkappaB and NFAT1 proteins. The increased expression of these transcription factors correlated with increased ERK/JNK, PKC and CN activities in these patients. Since activation of ERK/JNK/PKC kinases and CN phosphatase are required for stimulation of IL-2 transcription, these data provide a molecular explanation for the block in IL-2 production by M. leprae antigens. Thus the above study revealed suppression of all the three distinct biochemical pathways, viz. Ca-CN-NFAT pathway, PKC-NF-kappaB pathway, and MAPK-AP-1 pathway by M. leprae antigen (s) in anergized T cells of lepromatous patients which were activated by liposomal delivery of M. leprae antigens containing the two immunomodulators leading to optimal induction of IL-2 gene expression, which was required for the activation, and proliferation of T cells in lepromatous patients.

LABORATÓRIO CLÍNICO / DIAGNÓSTICO

Mendonça VA, Malaquias LC, Brito-Melo GE, Castelo-Branco A, Antunes CM, Ribeiro AL, Teixeira MM, Teixeira AL. Differentiation of patients with leprosy from non-infected individuals by the chemokine eotaxin/CCL11. Am J Trop Med Hyg 2007; 77(3): 547-50.

Diagnosis of leprosy is usually made clinically and there are no tests available for the routine laboratory diagnosis of the disease. The aim of this study was to investigate the potential role of chemokines as biologic markers of disease activity. We used an enzyme-linked immunosorbent assay to measure chemokines in plasma of patients with leprosy (LE) and non-infected (NI) individuals. There were significantly greater concentrations of the chemokines CCL3 and CCL11 in plasma of LE patients than in NI individuals. When the use of CCL11 to differentiate LE patients versus NI individuals was evaluated, the area under the receiver-operator-characteristic curve was 0.95 +/- 0.03 ($P < 0.0001$). In a group of selected individuals, CCL11 was useful in diagnosis of leprosy, thereby suggesting that measurement of this chemokine may be useful as an aid in diagnosing leprosis.

Nandan D, Venkatesan K, Katoch K, Dayal RS. Serum beta-glucuronidase levels in children with leprosy. Lepr Rev 2007; 78(3): 243-7.

A study was undertaken to find out the usefulness of determining the circulating levels of beta-glucuronidase, a lysosomal enzyme in leprosy affected children of less than 15 years of age. The serum enzyme levels were significantly higher in BB/BL patients compared to healthy control children as well as children with skin diseases other than leprosy. Treatment with Multidrug regimen advocated by WHO for multi/paucibacillary leprosy resulted in a significant fall in the serum enzyme levels in BB and BL cases. The findings suggest that serum beta-glucuronidase may be a useful parameter for the activity and extent of pathogenesis in leprosy.

MICOLOGIA

Reichart PA, Samaranayake LP, Bendick Ch, Schmidt-Westhausen AM, Jayatilake JA. Prevalence of oral Candida species in leprosy patients from Cambodia and Thailand. J Oral Pathol Med 2007; 36(6): 342-6.

BACKGROUND: Leprosy is a chronic bacterial infection which may lead to significant orofacial morbidity. However, reports on the oral mycotic flora of leprosy patients are rare. The aim of the current study was to explore the oral yeast carriage in two groups of leprosy patients. METHODS: 40 Cambodian (seven men, 33 women) and 48 Thai (14 men, 34 women) leprosy patients from Leprosy Rehabilitation Centre Khien Kleang, Phnom Penh, Cambodia and McKean Rehabilitation Center, Chiangmai, Thailand were randomly selected and their demographic data and clinical history were recorded. Tongue and palatal swabs of each patient were collected using sterile Fungi-Quick swabs (Hain Diagnostika, Nehren, Germany) and they were cultured aerobically on Sabouraud's dextrose agar and CHROMAgar (CHROMagar, Paris, France). Y east were identified by germ tube, chlamydospore production, and assimilation tests (API 20C AUX, Bio-Merieux, Marcy l'Etoile, France) and reconfirmed using APILAB Plus system (Bio-Merieux). RESULTS: Two groups (Cambodian and Thai) had median age of 35 and 64 years. They had been with leprosy for median durations of 17.7 and 38.9 years ($P<0.05$), respectively. Overall yeast carriage in two cohorts were 80% and 93.75%. Candida albicans had highest carriage rate in either group (65.6%, 44.4%). Candida krusei and C. glabrata existed as second-line colonizers after C. albicans. Candida glabrata carriage was significantly higher in Thai patients ($P<0.05$). Multispecies carriage was seen in three Cambodian (9.4%) and five Thai (11.5%) patients. CONCLUSIONS: This study indicates high oral yeast carriage in leprosy patients. Candida albicans remains predominant while C. krusei and C. glabrata are second-line oral colonizers. Co-inhabitation of multiple yeast species is also noted in these patients' oral mycotic flora.

MICROBIOLOGIA / BIOLOGIA MOLECULAR

Ribeiro-Guimarães ML, Tempone AJ, Amaral JJ, Nery JÁ, Gomes Antunes SL, Pessolani MC. Expression analysis of proteases of Mycobacterium leprae in human skin lesions. Microb Pathog 2007; 43(5-6): 249-54.

Proteases are commonly involved in bacterial pathogenesis and their inhibition has represented a successful therapeutic approach to treat infectious diseases. However, there is little information on the role of proteases in the pathogenesis of Mycobacteria. Five of these genes, three coding for putative secreted proteases, were selected in the present study to investigate their expression in Mycobacterium leprae isolated from skin biopsies of multibacillary leprosy patients. Via nested-PCR, it was demonstrated that mycP1 or ML0041, htrA2 or ML0176, htrA4 or ML2659, gcp or ML0379 and clpC or ML0235 are transcribed in vivo during the course of human infection. Moreover, the expression of Gcp in leprosy lesions was further confirmed by immunohistochemistry using a specific hyperimmune serum. This observat ion reinforces the potential role of mycobacterial proteases in the context of leprosy pathogenesis.

NEUROPATOLOGIA

Teles RM, Antunes SL, Jardim MR, Oliveira AL, Nery JÁ, Sales AM, Sampaio EP, Shubayev V, Sarno EM. Expression of metalloproteinases (MMP-2, MMP-9, and TACE) and TNF-alpha in the nerves of leprosy patients. J Peripher Nerv Syst 2007; 12(3): 195-204.

Matrix metalloproteinases (MMPs) and tumor necrosis factor alpha (TNF-alpha) play important and related roles in the pathogenesis of nerve injury. MMP-dependent and TNF-alpha-dependent processes of neurodegeneration, such as blood-nerve breakdown and immune cell recruitment, are characteristic of leprosy nerve damage. Our work has contributed to the understanding of the role of cytokines in the process, but the role of MMPs in the pathogenesis of neuritic leprosy has not been investigated. This study analyzed the changes in mRNA expression and immunodistribution of MMP-2, MMP-9, TNF-alpha-converting enzyme (TACE), TNF-alpha in nerves of 27 pure neuritic leprosy (PNL) patients, both acid-fast bacilli positive (AFB(+)) and acid-fast bacilli negative (AFB(-)), and 8 non-leprosy patients with control perip heral neuropathic conditions. MMP-2, MMP-9, and TNF-alpha mRNA expression was significantly induced in the AFB(-) relative to the AFB(+) neuritic leprosy group and nonlepritic controls; TACE levels were also elevated in the AFB(-) group, but this change was not statistically significant. Immunoreactive profiles for TNF-alpha and MMPs demonstrated strong reactivity of myelinated axons, infiltrating macrophages, Schwann cells, endothelial cells, and perineurial cells in neuritic leprosy biopsies. This study provides the evidence of the involvement of MMPs in the pathogenesis of PNL neuropathy.

Freitas MR. Infectious neuropathy. Curr Opin Neurol 2007; 20(5): 548-52.

[Ab] Resumo: PURPOSE OF REVIEW: Infectious neuropathy affects a large number of people worldwide. There is evidence of direct involvement of nerves by the infective agent, from the immune reaction of the patient or secondary to the toxicity of the drugs used during treatment. This group of neuropathies is often treatable or preventable. RECENT FINDINGS: There is a complex clinical picture of the neuropathy of leprosy, different pathological features and immunological mechanisms. If the skin is unaffected in leprosy it is not always easy to demonstrate that the neuropathy is due to leprosy. Peripheral neuropathy in patients with chronic infection with hepatitis C virus may be due to the virus, the development of vasculitis or direct neurotoxic effects of the treatment. Peripheral neuropathy has become the chief neurological syndrome in individuals infected with HIV-1. The antiretroviral therapies themselves can cause peripheral neuropathies clinically indistinguishable from those caused by the virus. The occurrence of chronic polyneuropathy as a late manifestation in Lyme disease is extremely rare and is not well understood. SUMMARY: Although infectious neuropathies are very frequent, mainly in developing countries, further studies are needed to elucidate their mechanisms of action, focusing on preventive interventions.

Cardoso CC, Martinez NA, Guimarães PE, Mendes CT, Pacheco AG, Oliveira RB, Teles RM, Illarramendi X, Sampaio EP, Sarno EM, Dias Neto E, Moraes MO. Ninjurin 1 asp110ala single nucleotide polymorphism is associated with protection in leprosy nerve damage. J Neuroimmunol 2007; 190(1-2): 131-8.

Leprosy is the major cause of non-traumatic neuropathy. Herein, we investigated the role of ninjurin 1, an adhesion molecule involved in nerve regeneration in leprosy. Our results demonstrated that M. leprae stimulates in vitro up-regulation of ninjurin mRNA in cultured Schwann and blood cells as well as in vivo mRNA and protein expression in leprosy nerve biopsies. A polymorphism (asp110ala) was investigated in a case-control study (1123 individuals) and no association was found with leprosy per se or with disseminated forms. Nevertheless, ala110 was associated with functional nerve impairment (OR=2.42; p=0.02 for ala/ala) and with lower mRNA levels. Our data suggests that asp110ala could be a valuable genetic marker of nerve damage in leprosy.

Job CK. Recent histopathological studies in leprosy, with particular reference to early diagnosis and leprous neuropathy. Indian J Lepr 2007; 29(2-3): 75-83.

In histopathological studies in leprosy, two important areas were identified in recently published work. They are early diagnosis and neuropathy. In histopathological examination, finding of M. leprae in tissues and/or granulomatous destruction of nerves are the two important findings to confirm the diagnosis. Immunopathological staining of M. leprae, PCR amplification of M. leprae antigen and S100 staining of Schwaann cells have considerably enhanced the sensitivity of histopathological diagnosis. If the two clinical findings such as hypopigmented patches with impaired sensation and thickened nerves accompanied by loss of sensation are the only ones that are taken into account for diagnosis, then a significant number of early patients will be missed. It is pointed out that biopsy examination of skin a nd nerves, when necessary, and skin-smear studies are indispensable diagnostic procedures. In the study of leprous neuropathy, there are several studies trying to decipher the entry of M. leprae into Schwann cells. The sharing of antigens between M. leprae and surface membrane of Schwann cells may be an important factor. However, there is much more to be learned in this area. In the control and prevention of neuritis, although corticosteroids administered along with multi-drug therapy was helpful, the benefit was not sustained.

Aung T, Kitajima S, Nomoto M, En J, Yonezawa S, Arikawa I, Goto M. Mycobacterium leprae in neurons of the medulla oblongata and spinal cord in leprosy. J Neuropathol Exp Neurol 2007; 66(4): 284-94

Peripheral neuropathy has been extensively studied in leprosy, a chronic disease caused by Mycobacterium leprae, but the central nervous system (CNS) is thought to be free from bacilli. Involvement of the CNS was explored in autopsy cases of clinically cured lepromatous leprosy (n = 67) and in non-leprosy cases (n = 15). Paraffin sections of the medulla oblongata and spinal cord were subjected to hematoxylin and eosin staining, Fite acid-fast staining, and anti-phenolic glycolipid-I (PGL-I) immunostaining. PGL-I-positive areas were microdissected from selected cases and nested polymerase chain reaction (PCR) targeting the M. leprae-specific repetitive sequence was performed. Of the 67 cases of leprosy, 44 (67%) had vacuolar changes of motor neurons either in medulla oblongata (nucleus ambiguous or hypo glossal nucleus) or spinal cord. Fite staining was negative, but PGL-I was positive in vacuolated areas. PCR revealed M. leprae-specific genomic DNA in 18 of 19 cases (95%) with vacuolated changes and 5 of 8 (63%) without vacuolated changes. All of above findings were negative in control cases. Terminal deoxynucleotidyl transferase dUTP nick-end labeling staining did not show a significant increase of apoptosis in the neurons. The PCR positivity had a significant correlation with PGL-I immunostaining ($p < 0.05$). The presence of vacuolar changes in the spinal cord was correlated with hand and feet deformity grades ($p = 0.04$). This study provides significant additional evidence to indicate that M. leprae is present in the CNS in a subset of patients. Further investigation is required to correlate this finding to motor dysfunction and silent neuropathy in leprosy.

OFATALMOLOGIA

Prasad NM, Prasad SR. Control of blinding eye diseases in leprosy: strategies for India. J Public Health Policy 2007; 28(4): 456-64.

Over half of the people affected by leprosy worldwide are in India, many of whom are severely disabled when they present for care. Changes in policy and the integration of the leprosy programme with general health services has given rise to new challenges for the control of blinding eye disease in leprosy patients. This paper looks at the challenges posed: management, materials, manpower, money, and mobility--all of which are common barriers to the availability, access, and utilization of services--and to monitoring existing programmes. We consider strategies to overcome these challenges and fulfill the goal of VISION 2020--eliminating avoidable blindness--through the provision of comprehensive eye care and strengthening the existing infrastructure. Formal cooperation between national governments, non-governmental organisations, and International Development Agencies will need to continue, although possibly, in different roles and with plans modified to be relevant to the local needs of leprosy patients.

Daniel E, Sundar Rao PS, Ffytche TJ, Chacko S, Prasanth HR, Courtright P. Iris atrophy in patients with newly diagnosed multibacillary leprosy: at diagnosis, during and after completion of multidrug treatment. Br J Ophthalmol 2007; 91(8): 1019-22.

To describe the prevalence and incidence of iris atrophy in patients with multibacillary (MB) leprosy. Methods and patients: Prospective longitudinal cohort study. 301 newly diagnosed patients with MB leprosy were followed up during the 2 years of treatment with multidrug therapy (MDT) and for a further 5 years with biannual ocular examinations. Incidence of iris atrophy was calculated as the number of patients with iris atrophy per person-year (PY) of follow-up among those who did not have iris atrophy at baseline. Stepwise multiple regression confirmed the presence of specific associations of demographic and clinical characteristics ($p<0.05$) with iris atrophy, detected by univariate analysis. RESULTS: Iris atrophy was present in 6 (2%) patients at enrolment. During MDT, with 445 PYs of follow-up , 9 patients developed iris atrophy (IR 0.02, 95% CI 0.01 to 0.04) that was associated with cataract (HR 15.13, 95% CI 3.71 to 61.79, $p<0.001$) and corneal opacities (HR 6.83, 95% CI 1.62 to 28.8, $p = 0.009$). After MDT, with 2005 PYs of follow-up, 60 patients developed iris atrophy (IR 0.03, 95% CI 0.023 to 0.039) that was associated with age (per decade; HR 1.40, 95% CI 1.10 to 1.78, $p = 0.006$), skin smear positivity (HR 3.50, 95% CI 1.33 to 9.24, $p = 0.011$), cataract (HR 3.66, 95% CI 1.85 to 7.25, $p<0.001$), keratic precipitates (HR 2.76, 95% CI 1.02 to 7.47, $p = 0.046$) and corneal opacity (HR 3.95, 95% CI 1.86 to 8.38, $p<0.001$). CONCLUSIONS: Iris atrophy continues to develop in 3% of patients with MB leprosy every year after they complete a 2-year course of MDT, and is associated with age, increasing loads of mycobacteria, subclinical inflammation, cataract and corneal opacity.

PATOLOGIA / HISTOPATOLOGIA / CITOLOGIA

Siddaraju N, Roy SK, Bundele MM, Badhe BA, Thappa DM. Fine needle aspiration cytologic diagnosis of erythema nodosum leprosum: a case report. Acta Cytol 2007; 51(5): 800-2.

BACKGROUND: Erythema nodosum leprosum (ENL), the type 2 lepra reaction occurring in lepromatous or borderline lepromatous leprosy, presents clinically with acute manifestations that compel the patient to seek medical attention. Recognition and timely management of these patients is critical in order to avoid permanent disability. Fine needle aspiration cytology (FNAC) is a simple, effective tool that aids in correct diagnosis and management of ENL. CASE: A 30-year-old woman presented with history of fever, reddening of the face, and multiple raised, reddish, painful swellings of the bilateral forearms and legs for 7 days. One year previously, she was diagnosed and treated for lepromatous leprosy with type 2 reaction. After a thorough clinical examination a diagnosis of ENL was made. FNA smears from the forearm swellings showed pus-like material with intact and degenerated polymorphonuclear leukocytes and many foamy macrophages with strong granular acid-fast bacillus (AFB) positivity. A cytologic diagnosis of ENL was given, which was confirmed on histopathologic examination of skin biopsy. CONCLUSION: Cytologic features such as a large number of intact and degenerated neutrophils with foamy macrophages and strong granular AFB positivity, in an appropriate clinical background, allows a confident diagnosis of ENL.

Miranda A, Amadeu TP, Schueler G, Alvarenga FB, Duppré N, Ferreira H, Nery JÁ, Sarno EM. Increased Langerhans cell accumulation after mycobacterial stimuli. Histopathology 2007; 51(5): 649-56.

To evaluate the role of Langerhans cells (LCs) in the local activation of leprosy lesions. LCs, acting as tolerance inducers and immune stimuli, are dendritic cells recently implicated in cutaneous homeostasis. The role of LCs in the defence against mycobacterial infection remains poorly understood. METHODS AND RESULTS: The number and distribution of CD1a+ skin cells and HLA-DR and intercellular adhesion molecule (ICAM)-1 expression were analysed in leprosy skin lesions and in delayed-type hypersensitivity (DTH) tests. The results showed a high number of LCs in tuberculin and lepromin tests, in tuberculoid lesions and in the epidermis and dermis during type I and II reactions. In multibacillary lesions, however, the number of LCs was consistently low in comparison with other groups. Increased num bers of LCs were accompanied by marked HLA-DR and ICAM-1 expression, suggesting a strong relationship between these immunological events. CONCLUSIONS: CD1a+ cells are implicated in the local immunological events taking place after mycobacterial stimuli and may account for the local activation of all types of reactional episodes in leprosy.

Vargas-Ocampo F. Diffuse leprosy of Lucio and Latapí: a histologic study. Lepr Rev 2007; 78(3): 248-60.

BACKGROUND AND PURPOSE: Ladislao de la Pascua described the spotted or lazarine leprosy for first time in 1844. Later on, Lucio and Alvarado studied and published it with the same names in 1852. Latapí rediscovered it in 1938 and reported it as 'Spotted' leprosy of Lucio in 1948. Frenken named it diffuse leprosy of Lucio and Latapí in 1963. Latapí and Chévez-Zamora explained that the fundamental condition of this variety of leprosy was a diffuse generalised cutaneous infiltration, naming it pure and primitive diffuse lepromatosis, upon which necrotising lesions develop, calling these lesions Fenómeno de Lucio or erythema necrotisans. A great number of histopathological reports have addressed the study of Lucio's phenomenon, and few about the histologic changes that take place in the course of diffuse lepromatous leprosy. The purpose of this work is to report the histologic findings observed in the study of 170 cutaneous biopsies of diffuse leprosy of Lucio and Latapí and 30 of Lucio's phenomenon. METHODS: This is a retrospective study, which included the examination of 200 biopsy skin specimens from 199 patients with diffuse leprosy at different course of the disease. These

cases were diagnosed in Mexico from 1970 to 2004. RESULTS: The histologic examination revealed a vascular pattern affecting all cutaneous vessels, characterised by five outstanding features: a) colonisation of endothelial cells by acid-fast bacilli, b) endothelial proliferation and marked thickening of vessel walls to the point of obliteration, c) angiogenesis, d) vascular ectasia, and e) thrombosis. Necrotising lesions seen in diffuse lepromatous leprosy displayed two histopathological patterns: one of them, non-inflammatory occlusive vasculopathy and, the other one, occlusive vasculopathy, leukocytoc lastic vasculitis, large neutrophilic infiltrate and lobular panniculitis. The first appeared as a result of the course of the occlusive vasculopathy produced by the colonisation of endothelial cells by Mycobacterium leprae. The second, as a result of a previous occlusive vasculopathy plus a leprosy reaction which is considered here as variant of ENL. CONCLUSIONS: Endothelial cell injury appears to be the main event in the pathogenesis of diffuse leprosy of Lucio and Latapí. Once M. leprae has entered the endothelial cell, the micro-organism damages the blood vessels, leading to the specific changes seen in this variety of lepromatous leprosy.

PSIQUIATRIA

Senturk V, Stewart R, Sagduyu A. Screening for mental disorders in leprosy patients: comparing the internal consistency and screening properties of HADS and GHQ-12. Lepr Rev 2007; 78(3): 231-42.

The aim of the study was to investigate the internal consistency and screening properties of the General Health Questionnaire-12 (GHQ-12) and the Hospital Anxiety and Depression Scale (HADS) in Turkish patients with leprosy. METHODS: The two screening instruments and a fully structured diagnostic interview (CIDI) were administered to 65 people drawn from all leprosy inpatient units in Turkey between March and June of 2001. The scales were evaluated using Cronbach's alpha and Receiver Operating Characteristics (ROC) analyses. For each scale, criterion validity was assessed for any psychiatric disorder, depressive disorder and anxiety disorder. RESULTS: The Cronbach alpha coefficients for the GHQ-12 and HADS were 0.84 and 0.81 respectively. For detecting any psychiatric disorder, the optimal c ut-off for the GHQ-12 was 4/5 (sensitivity 0.82, specificity 0.62). The optimal cut-off for the HADS total score was 12/13 (sensitivity 0.80, specificity 0.77). CONCLUSION: The results indicate that the General Health Questionnaire-12 and Hospital Anxiety and Depression Scale have satisfactory internal consistency, and performed well to a similar extent for detecting psychiatric disorders in leprosy patients.

QUALIDADE DE VIDA

Shen J, Liu M, Zhou M. Current situation of leprosy colonies/leprosaria and their future in P.R. China. Lepr Rev 2007 Sep; 78(3): 193-6.

OBJECTIVE: To identify the current situation of leprosy colonies/leprosaria and make some recommendations for improving the quality of life of people affected by leprosy in China. METHODS: A national survey using designed forms was carried out in 2004. The forms were filled in by local heads responsible for the management of leprosy colonies/leprosaria and sent to Provincial and National Centres for analysis. RESULTS: China had 605 leprosy colonies/leprosaria with 555 active leprosy patients (on treatment) and 18,175 ex-patients (people affected by leprosy) living in them at the end of 2004. Among 18,730 patients and people affected by leprosy, 13,430 (71.7%) had grade 2 disabilities. Among those with visible disability, 6392 (47.6%) lost the ability to take care of themselves due to serious deformity. Because of a decrease in health workers working at leprosy colonies and a shortage of medical materials, the health care quality of these people was neglected. Most colonies/leprosaria were located at remote and isolateS places with difficult transportation, and most buildings/houses were in danger of collapse because the colonies/leprosaria were built in the 1950's. Those affected by leprosy were in great need of help. CONCLUSION: The authors recommend that small, remote and isolated leprosy colonies should be closed. New leprosaria at District, Provincial or National levels should be established or some old leprosaria with good transportation should be reconstructed to house those affected by leprosy from closed leprosy colonies/leprosaria. The newly established or reconstructed leprosaria could act as centres for reference, training, rehabilitation and research on leprosy.

REABILITAÇÃO

Ishida Y, Shwe S, Win le L, Myint K. Needs assessment for income generation training of youths in leprosy families of a leprosy village in Myanmar. Nihon Hansenbyo Gakkai Zasshi 2007; 76(3): 197-206

After Myanmar eliminated leprosy in 2003, the prevention of disability (POD), as well as prevention of worsening disabilities (POWD) and rehabilitation became a new agenda, which is one of three national strategies of leprosy control beyond 2005. Since the training needs for income generation for youths living in leprosy villages were not well known, a small-scale survey was conducted in May 2005. This study found that the youths in Mayanchaung village, Yangon Division, were eager to receive training on income generation. After training they wanted to practice and improve their skills with the resources available, because they perceived that a short training course would not enable them to get a proper job. Although they were fully aware of income generation skills, they found it difficult to adequatel y consider issues such as resources for practicing skills after training, social marketing, and seeking job opportunities. They also felt that mediators could be helpful between villagers and external customers / retailers. On the other hand, the elders, most of whom had disabilities, wanted the youths to stay in the village to take care of them. A basic sewing and stitching training course that was planned to match the study results was produced in January 2006. After 11 months it was observed that a newly opened sewing workshop was busy operating 12 sewing machines because of a big order of making primary school uniforms. How effective the needs assessment was still unknown, but it was found that prior need assessment activities followed by a training course upon the real needs might promote the proper processes of social rehabilitation of youths in a leprosy village of Myanmar.

REABILITAÇÃO / CIRURGIA

Ishida Y, Lwin S, Myint K. Follow-up of tibialis posterior transfer surgery (TPT) for drop-foot in leprosy. Nihon Hansenbyo Gakkai Zasshi 2007; 76(3): 219-26.

Prevention of Disability (POD) service needs to be expanded for future reducing the leprosy burden. Tibialis Posterior Transfer Surgery (TPT) is an established procedure and relatively easy to do at district level general hospitals. It can protect further damages of affected foot and consequently reduce patient's social burden as well. Totally 70 TPT surgeries were done during a joint project of JICA on leprosy control and basic health service in Myanmar for training purpose (Jan/2002-Jan/2006). A follow-up assessment was done for exploring the effectiveness of foot drop surgery, in Nov/2006 at 9-selected townships in Mid-Myanmar. 33 cases (Male 22, Female 11) were reviewed and the mean of follow up period was 29.1 months (SD=7.1, 10-48 months). Total results were; good: 25 cases (76%), fair: 4 cases (12%) and poor: 4 cases (12%). In good and fair cases, patients were satisfied with the results and TPT improved the QOL of patients. In almost all cases (32/33, 97%) after TPT, patients are free from plantar ulcer. Most serious complication of operation (4 cases, 12%) identified was inversion deformity due to loosed tension of lateral tail of grafted TP tendon sutured to Extensor Digitorum Longus. From the results of TPT surgery follow-up, it can benefit much to the patients if resources permit to make it as a routine service in more places.

Menger DJ, Fokkens WJ, Lohuis PJ, Ingels KJ, Nolst Trenité GJ. Reconstructive surgery of the leprosy nose: a new approach. J Plast Reconstr Aesthet Surg 2007; 60(2): 152-62.

There has still been no reduction in the detection rate worldwide for leprosy, despite supervised multidrug therapy. In time, leprosy can result in a severe saddle-nose deformity leading to functional problems, disfiguration and stigmatization. In severe cases, only the nasal skin tissue and the lower lateral cartilages are preserved. In such cases, the ideal would be to restore the cartilaginous skeleton but, by contrast with other causes of saddle-nose deformities, this is complicated by the quantity and the poor quality of the remaining nasal mucosa. Leprosy-related saddle-nose deformities are therefore challenging and difficult to reconstruct with the techniques that have been proposed in the past. In this study, 24 patients underwent rhinoplastic surgery involving the use of autogenous costal and / or auricular cartilage or composite grafts. The nasal septum, the upper laterals and the anterior nasal spine were reconstructed with a dorsal onlay attached to a columellar strut with an extension on the proximal side. Before surgery, the saddle-nose deformities were classified according to severity with a new system based on clinical symptoms and signs. Postoperative evaluation was performed at least two years after surgery (N=17). Functional and aesthetic improvement, resorption rate, warping, infection and extrusion were analysed. Functional and aesthetic improvements were achieved in 15/17 patients. None of the patients developed an infection and extrusion or warping of the implants was not observed. The resorption rate depended on the localization and the type of cartilage implant. In general, auricular conchal cartilage implant grafts resulted in less resorption than costal cartilage. Least resorption (4/17 patients) was observed in the dorsal onlay grafts of both conchal (1/6) and costal cartilage grafts (3/11). Resorption of columellar strut implants and shield grafts was observed in 7/17 patients. No resorption was seen of composite grafts (0/4) and alar battens (0/7). Autogenous cartilage implants can be used to reconstruct saddle-nose deformities in leprosy with a minimum risk of complications. The preoperative grade of severity was used as a basis for the development of guidelines for optimal long-term functional and aesthetic outcome.

TERAPÊUTICA

Girdhar BK, Girdhar A, Chakma JK. Advances in the treatment of reactions in leprosy. Indian J Lepr 2007; 79(2-3): 121-34.

Morbidity in leprosy is almost always due to reactions. Similarly, to a great extent, deformities in leprosy are the consequence of reactions occurring both in borderline patients (type 1 or reversal reactions) and in lepromatous patients (type 2 or ENL reactions). Over the last three decades, work has centred around finding who are prone to getting the reactions, identifying the risk factors and improving the management of reactions in order to alleviate quickly the suffering and prevent and reverse nerve damage consequent to reactions. Though several new drugs have been tried and found somewhat useful, corticosteroids and thalidomide continue to be the mainstay in the management of leprosy reactions. A brief review of the current understanding is presented.

Samanta SK, Das D. Recent advances in ocular leprosy. Indian J Lepr 2007; 79(2-3): 135-50.

Proper MDT soon after detection of leprosy and anti-reaction measures with newer steroids, regular supervision and monitoring of those released from treatment (RFT) reduce the incidence of ocular leprosy to a remarkable extent. Today, most eye complications are because of normal ageing process or of other phenomena in normal healthy population. Cataract and lagophthalmos are the main causes of blindness. However, in India, though the rate of cataract surgical coverage is up to the mark, the same for lagophthalmos is lagging far behind. Integration of management of ocular leprosy into community eye health care service is the talk of the day along with other health care facilities delivered to people affected with leprosy (PAL). Routine eye examinations are necessary for all PB and MB patients, as well as for the RFT persons in order to detect and treat eyes that are at high risk. All eye surgeries can be performed when needed, irrespective of deformities and bacteriological status, by latest microsurgical techniques with good outcome, and better rehabilitation measures. Reorientation training in ocular leprosy is the immediate special need for ophthalmologists, paramedical ophthalmic assistants and eye health care managers working in general hospitals in those areas that were previously [quot]leprosy endemic zones[quot].

Biosca G, Casallo S, López-Vélez R. Methotrexate treatment for type 1 (reversal) leprosy reactions. Clin Infect Dis 2007; 45(1):7-9.

Corticosteroids are the drugs of choice for treatment of type 1 leprosy reactions, but when these agents cannot be used because of their adverse effects, alternative treatments are needed. We report the first case, to our knowledge, of a type 1 leprosy reaction that was successfully treated with methotrexate in a patient intolerant to corticosteroids who had borderline lepromatous leprosy.

TERAPÊUTICA / CONTROLE

Ebenso J, Ebenso BE. Monitoring impairment in leprosy: choosing the appropriate tool. Lepr Rev 2007; 78(3): 270-80.

OBJECTIVE: To assess to what extent the Maximum WHO Impairment Grade, the EHF Score and Impairment Summary Form (ISF) reflect changes in impairment, both in number(s) and severity. DESIGN: The impairment data at registration and at release from treatment of 444 persons affected by leprosy registered for MDT from 1994-2003 in Federal Capital Territory, Nigeria were analysed using three monitoring tools. RESULTS: Of the 444 patients, 92 people had a change (10 deteriorated, 70 improved while 12 had improvement in some parts of their body and deterioration in other parts) in their impairment between Registration and Release from Treatment. Of the 10 people whose impairment status deteriorated, the WHO Grade missed 7 and the EHF score missed 4. The ISF missed none. Of the 70 whose impairment status improve d, the WHO grade missed 27; the EHF score missed 20 and the ISF missed 9. The WHO Grade had a sensitivity of 50%, the EHF Score 61% and the ISF 90%. Negative predictive values were 88%, 91% and 98% respectively. CONCLUSIONS: The Maximum WHO Impairment Grade use should be limited to an indicator of late case detection. The EHF score is better used at programme level than individual patient level. The ISF is a sensitive tool for monitoring impairments at patient level to aid clinical decision making.

TERAPÊUTICA / REAÇÕES ADVERSAS

Deps PD, Nasser S, Guerra P, Simon M, Birshner RC, Rodrigues LC. Adverse effects from multi-drug therapy in leprosy: a Brazilian study. Lepr Rev 2007; 78(3): 216-22.

INTRODUCTION: The WHO MDT for leprosy treatment was officially introduced in Brazil in 1991 and comprises three drugs: dapsone, rifampicin and clofazimine. There are few good studies on the frequency of side-effects attributable to MDT in Brazil. METHODS: A retrospective and descriptive study carried out in a LCP in Vitória, State of Espirito Santo, Brazil. A specific and detailed protocol about side-effects was prepared and filled in from the patient records. RESULTS: One hundred ninety four patients' records were analysed looking for side-effects attributable to MDT. Side-effects were attributed to at least one MDT component in 88 (45%) patients and 85 had side-effects due to dapsone, 24 due to rifampicin and 18 due to clofazimine. 185 episodes were identified. The suspected drug was stopped in 47 out of 88 episodes (24% patients); 46 had dapsone stopped, 5 had rifampicin stopped and no-one had clofazimine stopped. CONCLUSION: Side-effects attributed to MDT is more frequent than previously described, resulting in interruption of treatment in many patients.

Walker SL, Waters MF, Lockwood DN. The role of thalidomide in the management of erythema nodosum leprosum. Lepr Rev 2007; 78(3): 197-215.

Erythema nodosum leprosum (ENL, Type 2 reactions) complicates lepromatous and borderline lepromatous leprosy and can affect many organ systems, often with irreversible damage. The reactions commonly occur in the 2 years after starting treatment and often run a recurrent or chronic course, sometimes for many years. Even with WHO multi-drug therapy about 30% of LL patients experience ENL. We review drug management of ENL focussing on data from controlled trials and other studies. The treatment of ENL is difficult because high doses of steroids may be required for prolonged

periods and do not always control the inflammation. The paradox of ENL is that it can be a life-threatening disorder and requires control with immunosuppression which may itself pose life-threatening risks for patients. Treatment with thalidomide provides an effective alternative to steroid therapy, gives better long-term control and avoids the adverse effects of prolonged steroid therapy. Controlled clinical trials have demonstrated that thalidomide rapidly controls ENL and is superior to aspirin and pentoxifylline. However, thalidomide is teratogenic when taken in early pregnancy and is unavailable in many leprosy endemic countries. We discuss the role of thalidomide in treating ENL, the complications encountered and risk reduction strategies that can be used. These include good patient selection and counselling, close supervision and adequate access to appropriate contraception. Further research is needed to improve the understanding and treatment of this severe and debilitating complication of leprosy. Topics for research include: i. The development of validated tools to measure the severity and/or activity of ENL. ii. A detailed assessment of the neurotoxic effects of thalidomide when used to treat EN L. iii. A well designed trial comparing thalidomide with prednisolone. iv. The development of a safe and effective alternative to both steroids and thalidomide.

Ozaki M, Ishikawa M. [Long-term follow-up of ofloxacin-combined therapy for leprosy patients]. Nihon Hansenbyo Gakkai Zasshi 2007; 76(3): 207-18.

This reports a long-term follow-up study on clinical effects of ofloxacin (OFLX)-combined therapy to 14 leprosy patients with various types and stages. Combined drugs were diaminodiphenyl sulfone (DDS), rifampicin (RFP) and clofazimine. Clinical evaluation of the treatment after OFLX-combined therapy was remarkable improvement 10 cases, improvement 3 and re-exacerbated after improvement 1 to whom clofazimine and minocycline were prescribed. The evaluation during the follow-up was remarkable improvement 10, improvement 1; three cases died of traffic accident or complications not related to chemotherapy and none of them showed relapse of leprosy. Bacterial negativity after onset of OFLX-combined therapy was achieved in about the same periods as RFP-combined therapy. OFLX-combined therapy was effective an d safe. This follow-up study supports the efficacy of clinical guideline for the use of new quinolones published by Japanese leprosy Association.