

# Literatura Corrente

## Current literature

### Aspectos Psicossociais

Tsutsumi A, Izutsu T, Islam MD, Amed JU, Nakahara S, Takagi F et al. Depressive status of leprosy patients in Bangladesh: association with self-perception of stigma. *Lepr Rev* 2004; 75(1):57-66.

Stigmatization by the general population and their negative attitudes towards leprosy negatively impacts on patients' mental health, and so too does patients' perception of that stigma. The objective of this present study is to assess the depressive status of leprosy patients, the patient perception of that stigma, and its association with their depressive status in Dhaka, Bangladesh. Subjects were 140 patients, and a selected comparison group of 135 local people without any chronic diseases. To evaluate depressive status, the Center for Epidemiologic Studies Depression scale (CES-D) Bengali version was applied. The patient group's depressive status was significantly more severe than that of the comparison group. Depressive status of those who answered affirmatively was significantly more severe than that of those who answered negatively for three responses to questions: 1) 'I have been physically attacked by people', 2) 'I feel people regard me as strange' and 3) 'I have been refused the purchase of something by a shopkeeper'. The results showed that the depressive status in leprosy patients was greater than that of the general public. Further, actual experiences of discrimination based on stigma associated with the depressive status of leprosy patients. Mental health care for patients, regulation of discriminatory action and education that would decrease social stigma among the general population, especially people who might often have contact with patients, seem necessary to improve the mental health of Bangladeshi leprosy patients.

### Biologia Molecular

Haile Y, Ryon JJ. Colorimetric microtitre plate hybridization assay for the detection of *Mycobacterium leprae* 16S rRNA in clinical specimens. *Lepr Rev* 2004; 75(1):40-9.

We have developed a colorimetric microtitre plate hybridization assay in order to simplify detection of

*Mycobacterium leprae* in clinical specimens. This system detects the products amplified by a sensitive RT-PCR assay targeting a species-specific sequence of the bacterial 16S rRNA. The assay detected as few as 10 bacilli isolated from infected nude mouse lymph nodes or human skin biopsies. Sensitivity for diagnosis of clinical specimens was assessed for 58 tissue biopsies from untreated leprosy patients. The assay detected *M. leprae* RT-PCR products in 100% of biopsies from patients with multibacillary disease and 80% of biopsies from patients with paucibacillary disease, for an overall sensitivity of 91.3%. The test was highly specific as no RT-PCR products were amplified from skin biopsies of normal individuals or patients with skin diseases other than leprosy. The colorimetric assay is faster, more sensitive, and simplifies detection of RT-PCR products compared to Southern blot analysis. It may be useful for diagnosis of difficult cases of leprosy, and, since RNA is rapidly degraded after cell death, it may be appropriate for assessing response to therapy and for distinguishing relapse from reaction.

Maeda Y, Brennan PJ, Makino M. Studies of lipoproteins of *Mycobacterium leprae*. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):15-21.

The deciphering of the genomic sequence of *Mycobacterium leprae* has made possible to predict the possible lipoproteins. The consensus sequence at the N-terminal region of the protein, including the cysteine residue to which the lipid moiety gets attached, provides a clue to the search. As such, more than 20 putative lipoproteins have been identified from *Mycobacterium leprae* genomic sequence. Lipoprotein LpK (Accession no. ML0603) which encodes for 371 amino acid precursor protein, was identified. Expression of the protein, in *Escherichia coli* revealed a 33 kD protein, and metabolic labeling experiments proved that the protein was lipidated. The purified lipoprotein was found to induce production of IL-12 in human peripheral blood monocytes which may imply that *M. leprae* LpK is involved in protective immunity against leprosy. Pursuit of such lipoproteins may reveal insights into the pathogenesis of the disease.

Sunderkötter CH, Tomimori-Yamashita J, Nix V, Maeda SM, Sindrilaru A, Mariano M, Sorg C, Roth J. High expression of myeloid-related proteins 8 and 14 characterizes an inflammatorily active but ineffective response of macrophages during leprosy. *Immunology* 2004; 111(4):472-80.

Macrophages are decisive cells for the course of leprosy as they phagocytose *Mycobacterium leprae* and have the potential to influence the specific immune response. Expression and release of the myeloid-related protein (MRP) 8 and MRP14 (S100A8 and S100A9) characterize a proinflammatory subtype of macrophage that is prominent in, for example, murine infection with lack of a T helper 1 cell response and in certain highly active chronic inflammations of mice and humans. We investigated cutaneous biopsies of the different forms of leprosy (41 untreated patients) including leprosy reaction type 1 (reversal reaction) and type 2 (erythema nodosum leprosum) (n = 18) for expression of MRP8 and MRP14 by subtypes of macrophages. Concomitantly we determined serum levels of MRP8 and MRP14 by sandwich enzyme-linked immunosorbent assay. Expression of MRP8 and MRP14 by CD68-positive macrophages was low in tuberculoid leprosy and rose significantly in borderline tuberculoid leprosy and especially in multibacillary forms, there being expressed by mycobacteria-loaded foam cells. A significant rise of MRP8 and MRP14 expression also occurred in lepra reactions compared to the corresponding non-reactional forms. In type 2 reactions this additional increase was associated with a significant elevation of serum levels. In type 1 it was associated with expression of MRP8 and MRP14 by epitheloid and giant cells, which so far were considered not to express both proteins. In conclusion, we present evidence that the two prominent proteins MRP8 and MRP14 can be re-expressed in vivo by tissue macrophages in chronic infection, that their increased expression is characteristic for a macrophage subtype associated with high inflammatory but low antimycobacterial activity in the absence of a T helper 1 response, and that their significant rise in serum during erythema nodosum leprosum bears diagnostic and pathophysiological relevance.

Williams DL, Oby-Robinson S, Pittman TL, Scollard DM. Purification of *Mycobacterium leprae* RNA for gene expression analysis from leprosy biopsy specimens. *Biotechniques* 2003; 35(3):534-6, 538, 540-1.

Gene expression analysis in *Mycobacterium leprae*, an obligate intracellular pathogen and the etiologic agent of leprosy, has been hampered by the lack of an efficient method to purify RNA from leprosy lesions. Therefore to date,

transcripts for only a few genes have been identified. We report the use of a single-tube homogenization/RNA extraction method that produces enough RNA to study the expression of 30 genes from a single skin biopsy specimen of a multibacillary leprosy patient and demonstrate that RNA can be purified after fixation of biopsies in 70% ethanol for up to a year. This represents a major advancement in the ability to study *M. leprae* gene expression directly from biopsy material and should help to define genes that are associated with intracellular survival of this human pathogen.

## Biologia Molecular/ Epidemiologia

Izumi S, Agusni I. [Global situation of leprosy and recent progress in molecular epidemiology of the disease]. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):37-46.

Recent discovery of genetic diversity of *Mycobacterium leprae* such as variable number of tandem repeats opened a new era in molecular epidemiology of leprosy infection. It was revealed that the leprosy bacillus in residential environment of endemic villages is an important source of infection. The global elimination strategy will be revised taking new molecular epidemiological knowledge into account. Responsibility of leprosy specialist is to propose feasible control program to local administration based on the epidemiological analysis on transmission of the disease.

Matsuoka M, Zhang L. [Molecular epidemiology of the leprosy]. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):7-14.

Application of molecular biological techniques to the epidemiological study of leprosy is described. Studies of detecting *Mycobacterium leprae* DNA in samples of the nasal mucus are discussed in terms of the epidemiology and the significance of high prevalence. Epidemiological studies on the transmission of leprosy and correlation between geographic distribution of different *M. leprae* rpoT genotypes and prehistoric spread of the leprosy by genotyping based on the genomic polymorphism are introduced.

## Clínica

Daniel E, Duriasamy M, Ebenezer GJ, Shobhana, Job CK. Elevated free tear lactoferrin levels in leprosy are associated with Type 2 reactions. *Indian J Ophthalmol* 2004; 52(1):51-6.

Purpose: To determine the association of demographics, leprosy and ocular characteristics with altered levels of lactoferrin in the tears of normal subjects and leprosy patients,

and to detect the presence of antibodies to lactoferrin in these tear samples. Method: We collected light-stimulated tears from 298 leprosy patients and an equal number of normal subjects using the glass capillary method. Free lactoferrin levels were estimated using ELISA and the presence of antibodies to lactoferrin was detected using the immuno-blotting method. Significant associations were looked for between tear lactoferrin levels and demographic characteristics, leprosy characteristics such as type of disease, duration of disease, reactions, deformity and bacterial load, and ocular complications, using chi-square and regression analysis. Results: Tear lactoferrin levels with a mean (SD) of 2.55 (2.83)mg/ml in the control group were significantly different ( $P<0.000$ ) from leprosy patients with a mean (SD) of 5.66 (7.21)mg/ml. Age showed an inverse correlation with tear lactoferrin levels in controls. Increased bacterial load, grade 2 leg deformity and Type 2 reactions were significantly associated ( $P<0.05$ ) with increased tear lactoferrin levels. Type 2 reactions remained significantly associated ( $P=0.01$ ) on multiple regression analysis. Tear lactoferrin levels were not associated with gender, serum lactoferrin levels, Type 1 reactions, face patches, treatment status, orbicularis oculi weakness, lagophthalmos, ectropion, entropion, corneal opacity, cataract and iridocyclitis. Conclusion: Age is inversely related to tear lactoferrin levels in normal subjects. Free lactoferrin levels in tears are significantly higher in leprosy patients compared with normal controls. Type 2 reactions in leprosy are significantly associated with elevated tear lactoferrin levels.

Dave S, Thappa DM, Nori AV, Jayanthi S. A rare variant of erythema nodosum leprosum: a case report. *Dermatol Online J* 2003; 9(5):11.

We report a patient with lepromatous leprosy who developed a rare variant of type-2 lepra reaction, characterized by pustular lesions, on switching from WHO multi drug therapy (MDT) to ofloxacin-aided MDT.

Ghorpade A. Reactional tattoo inoculation borderline tuberculoid leprosy with oedematous tattoos. *Lepr Rev* 2004; 75(1):91-4,.

Tattoo inoculation borderline tuberculoid (BT) leprosy in upgrading reaction with prominent tattoo oedema developing after starting paucibacillary multidrug therapy (PB MDT) is reported. The diagnosis was confirmed by histopathology. An excellent response to oral steroids and PB MDT was seen. There is only one similar report in the literature.

Rodríguez, Gerzaín. Adenopatías generalizadas como presentación de la reacción leprótica tipo 2 / Generalized adenopathy as a manifestation of type 2 reactional leprosy. *Biomédica (Bogotá)*; 2003; 23(4):373-387.

Las reacciones en los pacientes con lepra son manifestaciones clínicas graves de inflamación aguda en las lesiones crónicas del enfermo, capaces de producir daño irreversible e incapacitante. Estudiamos un hombre de 46 años con reacción leprótica tipo 2, que consultó por fiebre, malestar general, sensación de obstrucción nasal, nódulos cutáneos y adenopatías generalizadas. El cuadro hemático mostró leucocitosis con neutrofilia. Entre varios diagnósticos clínicos sugeridos no se pensó en lepra. Una biopsia ganglionar demostró necrosis extensa del órgano que estaba infiltrado por polimorfonucleares y macrófagos espumosos, con necrosis de vénulas y depleción linfocítica. No se hizo coloración de ZN, pero sí de Gomori, que tiñó muy bien los bacilos de Hansen, pero no se detectaron por el patólogo, que no hizo un diagnóstico concluyente. Veinte meses después, el paciente presentó síntomas semejantes con adenopatías generalizadas y nódulos cutáneos más numerosos, la biopsia de uno de los cuales demostró lepra lepromatosa con eritema nodoso leproso o reacción tipo 2. El tratamiento antileproso con poliquimioterapia y antirreaccional con talidomida curó al paciente, que 3 años después no presenta secuelas, pese a los 20 meses transcurridos para precisarse el diagnóstico. Comentamos este caso clínico y revisamos los factores predisponentes, la histopatología, los diagnósticos diferenciales de la adenopatía leprosa, la patogenia, el pronóstico y el tratamiento de la reacción tipo 2 en lepra, que constituye una urgencia médica, capaz de originar incapacidad grave y que, como en este enfermo, puede cursar con adenopatías como signos y síntomas predominantes.

## Epidemiología

Dony JF, Ahmad J, Khen Tiong Y. Epidemiology of tuberculosis and leprosy, Sabah, Malaysia. *Tuberculosis (Edinb)* 2004; 84(1-2):8-18.

The objectives in this epidemiology review are to measure and report the extent of morbidity and mortality due to tuberculosis (TB), the proportion of new sputum smear positive cases in districts and the status of cohort analysis as of 1999. As for leprosy, the main objective is to determine morbidity and the treatment outcomes of Multiple Drug Therapy (MDT). Based on the results obtained, a comprehensive action plan for prevention, control and monitoring of tuberculosis and leprosy cases and patients is being produced and implemented throughout the state. The

analysis concentrated on patients diagnosed at all out-patient units and admitted in all of the state's hospitals. The patient particulars were recorded using a standardized format based on TB and Leprosy Health Management Information System (TB HMIS). TB was the second highest by notification of communicable diseases in Malaysia in 2001. 29% or about one-third of the national TB cases are from Sabah. However, it has been noted that there was an average decline of 2.6% in annual notification since 10 years ago to date. There was also a reduction of 11.4% in 2001 as compared to annual notification in 2000. Immigrants contribute more than 24% in detection of new cases since 1990. Treatment success rate in term of completion of treatment to date is 82%. Mortality rate has steadily declined from 14 deaths to 7 deaths per 100,000 population. Leprosy in Sabah also contributes to 30% of the yearly total caseload of Malaysia and has the highest notification rate of 2 per every 100,000 population as compared to other states. The average registered leprosy cases over the past 5 years are 239 cases and the prevalence rate is 0.7/10,000 population. The state has successfully achieved its goal to decrease leprosy as per the World Health Organization (WHO) goal of yearly overall prevalence rate of less than 1 case for every 10,000 population. However, the districts of Kudat, Tawau, Lahad Datu, Kota Kinabalu and Semporna are still within the prevalence rate of more than one per 10,000 population. This review highlights some interesting findings which can be incorporated into the State and Districts action plans and strategies. It is also noted that in order to translate National Plans and Strategies into effective action at the community level, health workers need relevant up-to-date knowledge of the pattern of health and disease, and of their determinants, in each district. The Sabah Health Department continues to organize and support programs related to management and control of tuberculosis and leprosy to progressively reduce the incidence of these diseases in the community by breaking the chain of transmission of *Mycobacterium tuberculosis* and *M. leprae*, respectively.

Kalk A, Fleischer K. The decentralization of the health system in Colombia and Brazil and its impact on leprosy control. *Lepr Rev* 2004; 75(1):67-78.

Decentralization policies are an integrated component of health sector reform in an increasing number of countries. The ability of such policies to improve the health system's quality and efficiency is backed up by limited scientific evidence. This study intends to evaluate the impact of decentralization on a specialized field of disease control (leprosy control) in Colombia and Brazil. It analyses the respective juridical base, epidemiological indicators and local

publications. Furthermore, 39 semi-structured interviews with key informants were conducted. In both countries, the devolution of technical responsibility and financial resources to the municipalities was the implemented form of decentralization. Access to preventive and curative health care and the community participation in decision-making improved clearly only in Brazil. The decentralization to private providers in Colombia had dubious effects on service quality in general and still more on public health. The flow of finances (including finance collection through state-owned taxes instead of insurance companies) seemed to be better controlled in Brazil. Leprosy control in Brazil took advantage of the decentralization process; in Colombia, it came close to a collapse.

Meima A, Richardus JH, Habbema JD. Trends in leprosy case detection worldwide since 1985. *Lepr Rev* 2004; 75(1):19-33.

Trends in case detection and case detection rate (CDR) since 1985 are described at regional and national levels. Annual case detection by WHO Region was available for 1994-2000. Using different sources, complete time series for case detection were constructed for 1985-1998 for a group of 33 endemic countries cumulatively (top 33), and for 14 individual countries (top 14). Population statistics were used to derive CDRs. India contributed 79% to global case detection in 1998. Africa, the Americas and South-East Asia each contributed about 30% when India is excluded. During 1994-2000, case detection did not decrease in these three WHO Regions. The 33 countries contributed 99% and 98% to global case detection in 1994 and 1998, respectively. Cumulative case detection for the top 33 minus India gradually increased, overall almost doubling. The contribution of the top 14 to case detection of the top 33 hardly changed over time, equalling 96% in 1998 (81% when India is excluded). In terms of annual case detection, Brazil was always ranked second after India; it accounted for 27% of 1998 case detection in the top 33 except India. In 1998, seven of the top 14 countries including India and Brazil had CDRs above 2 per 10,000. The CDR did not exceed 1 per 10,000 for the other half. Decreasing tendencies in CDR, either for the whole period or in the 1990s, are observed for four of the top 14 countries (Guinea and three Western Pacific countries: China, Vietnam and the Philippines). In conclusion, there is no general decline in case detection to date, and several important countries still have high CDRs. Prevalence is an irrelevant indicator for monitoring epidemiological changes in leprosy. Trends in the transmission and incidence of leprosy are still completely unclear, necessitating further research. The target to eliminate leprosy

as a public health problem, defined as a prevalence of less than 1 per 10,000, is therefore also an inadequate yardstick for decision making on leprosy control.

Norman G, Joseph GA, Udayasuriyan P, Samuel P; Venugopal M. Leprosy case detection using schoolchildren. *Lepr Rev* 2004; 75(1):34-9.

An innovative method that combined awareness creation with screening of high school students by their peers was undertaken in 26 randomly selected schools in the project area of the Schieffelin Leprosy Research and Training Center, Karigiri, Vellore, India. This method entailed educating teachers and student leaders in grades 8-12 about leprosy and how to suspect leprosy among their peers. The student leaders in turn conducted a similar awareness programme for their peers and encouraged them to report if they suffered from any skin problem or skin lesion. Based on the reporting by their peers, the class leaders prepared a 'suspect list'. Within a fortnight of the awareness program, a trained leprosy worker visited the school and examined all the students on the 'suspect list'. Those diagnosed to have leprosy were referred to a medical officer, who then confirmed the diagnosis and initiated treatment. Among the 23,125 students enrolled in the 26 randomly selected schools, 234 student leaders were educated about leprosy and trained to detect suspect lesions among their peers. A total of 2200 (9.5%) children reported with skin lesions to their leaders and after screening by a leprosy supervisor and confirmation by a medical officer, 14 new cases (NCDR 6.05/10,000) were detected. This rate was found to be comparable with case detection rates of annual school surveys done during the National Leprosy Eradication Programme (NLEP), when all schoolchildren were examined. The paper suggests that schoolchildren can be used effectively in leprosy case detection and this method has the additional advantage of creating awareness among them, their teachers and communities.

Saha K, Kapoor L, Arora VM, Chattopadhyaya D. Malaria, leprosy and dapsone. *Southeast Asian J Trop Med Public Health* 2003; 34(3):501-4.

Although the preventive action of dapsone against *P. falciparum* malaria was known for many years, there was no report about the incidence of *P. falciparum* malaria in leprosy patients treated with dapsone, especially from areas of Southeast Asia where both leprosy and malaria are endemic. Therefore, two clinic-based malaria surveys were undertaken at a gap of 12 years, comprising 506 lepromatous leprosy

patients and 499 febrile nonleprosy control subjects. Both the surveys showed that the lepromatous patients treated with MDT had only *P. vivax* malaria (incidence comparable to the febrile nonleprosy controls) with complete freedom from *P. falciparum*. On the contrary, control subjects not taking any-leprosy drugs and staying with the leprosy patients at the same beggars' home, had both *P. vivax* and *P. falciparum* malaria. It is postulated that dapsone provided protection against *P. falciparum* among leprosy patients.

## Epidemiologia e Controle

Ratre HL, Bansal AK, Varma SS, Jha A. Cleaning of leprosy case registers in Chhattisgarh. *Indian J Public Health* 2003; 47(4):180-2.

Cleaning of register exercise was based on examination of records and not of patients. Because of this exercise, names of 5676 patients were deleted for various reasons viz. completed FDT, Defaulter, AMDT etc. The findings of the present study are more or less similar to the finding of similar other studies carried out in different countries from time to time. Updating of leprosy registers should be a routine activity. For this sensitization of health functionaries at various level must be carried out about importance of cleaning of register and for systematic effort to review the registers at least on sample basis.

## Genética

Gorodezky C et al. Molecular mechanisms of MHC linked susceptibility in leprosy: towards the development of synthetic vaccines. *Tuberculosis (Edinb)* 2004; 84(1-2): 82-92.

Tuberculoid (TT) and lepromatous leprosy (LL) develop in the human host depending on his ability to trigger a specific cellular immune response (CIR). Different genes have been demonstrated in susceptibility/protection and may explain the forms of leprosy. The major histocompatibility complex (MHC) play an important role. The aim of the study was to explore the contribution of human leukocyte antigen (HLA) DRB1, DQA1, DQB1 and DQ promoter genes in LL Mexican patients. Six families (26 LL, three TT patients and 27 controls) were analyzed; 114 unrelated patients were compared with 204 controls. Class I typing was done by the standard microlymphocytotoxicity and class II typing using PCR-SSOP. Haplotype segregation correlated with specific CIR in vivo and in vitro using lepromin. Haplotype sharing was significantly deviated in the affected sibs ( $p=0.01$ ). Six healthy sibs were non-responders to lepromin and four of them were DQ1 homozygotes. DQ1 was significantly associated with LL and with

non-responders. We set up macrophage activation experiments after infecting these cells with  $5 \times 10^6$  bacilli to demonstrate if elimination occurred in the context of DQ1. When DQ1 was present on macrophages and on T cells, bacteria were poorly eliminated from the cell (32%) while when absent, 76% of the individuals were able to eliminate the bacilli ( $p=0.03$ ). DRB1\*1501 DQA1\*0102-DQB1\*0602 (DQ1 subtype) was significantly increased in the patients, indicating its participation in susceptibility. QBP 5.11/5.12 promoter present in the mentioned haplotype, and QAP 1.4, linked to DRB1\*1301/02 haplotypes were also associated. Two mechanisms are suggested: the promoter polymorphisms may influence allele expression and thus the amount of peptides presented to the T-cell receptor, leading to a deficient CIR: HLA restriction is important for vaccine design; the way peptides anchor the DRB1\*1501 groove may be relevant to the activation of TH1 cells, which contribute to an efficient presentation of peptides inducing a protective T-cell response.

## História

George AO, Daramola OO. Dermatology in Nigeria: evolution, establishment and current status. *Int J Dermatol* 2004; 43(3):223-8.

The history of dermatology in Nigeria can be summarized by listing the "first events and people" who played major roles. The first dermatologist to work in Nigeria was George HV Clarke in the mid 1950s. He was based in Lagos. Organized training/teaching and research in dermatology was first established at the University of Ibadan in the western region of Nigeria, in collaboration with the Institute of Dermatology, London. The first set of dermatologists from the Institute on secondment who established dermatology as a discipline at the University of Ibadan were Roger RM Harman as lecturer and GC Wells as the Visitor/supervising consultant in 1962. The first indigenous dermatologist was Anezi Okoro. The first female dermatologist was Yetunde M Olumide. The first (and still the only) Department of Dermatology was at the Obafemi Awolowo University in Ile-ife in the western region of Nigeria, started by a German trained dermatologist: F. Soyinka, the junior brother of Nigeria's first (and only) Nobel Laureate. The first leprologist in Nigeria was George Stanley Browne, a medical missionary. He worked briefly as an associate lecturer (1963-65) under Professor Alexander Brown at the University of Ibadan. Most of the events in Nigeria's dermatology history as well as the greatest concentration of dermatologists in the country have been around the south-western part of the country: Lagos, Ibadan, and Ile-ife. The military coup and the political events that occurred thereafter had a great negative impact on the

development/growth of dermatology in Nigeria. The documentation by Ryan as of 1990 indicates that many African countries still do not have a single trained dermatologist.

Gormus BJ, Martin LN, Baskin GB. A brief history of the discovery of natural simian immunodeficiency virus (SIV) infections in captive sooty mangabey monkeys. *Front Biosci* 2004; 9:216-24.

Experimental leprosy studies using *Mycobacterium leprae* inoculum isolated from a sooty mangabey monkey (SMM) resulted in the accidental discovery that SMM's asymptotically carry simian immunodeficiency virus (SIV) that is pathogenic in macaques. We showed that the SMM virus, SIVDelta, was antigenically related to SIVmac, which had been identified in macaques, and to the human immunodeficiency virus (HIV). Similar asymptomatic natural SIV infections had been reported in African green monkeys (AGM). Our results together with observations of others led us to propose that both SIVmac and SIVDelta originated in SMM and that SIV emerged in humans as a result of early African nonhuman primate SIV trans-species infections in humans.

Mori S, Kato S, Yokoyama H, Tanaka U, Kaneda S. [The history of Yunosawa village and the policy of leprosy of Japan. IV]. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):47-63.

There was a village which was called Yunosawa, lots of leprosy patients lived, existed from 1887 to 1941, Kusatu town, Gunma Prefecture, Japan. It was the only place continued securing self-government to the last as area was free from the isolation policy of State in prewar days there. The aim of this study will make clear the dynamism of "The protection from the tension of the society of leprosy patient currently persecuted" to "The defense of the society from the leprosy patient who is a source of infection". In this study, explained the factor of confusion to a National Leprosarium Kuryu Rakusen-en during World War II and considered relation between patient movement and residents of Yunosawa village at the postwar period.

## Imunologia

de la Barrera S et al. NK cells modulate the cytotoxic activity generated by *Mycobacterium leprae*-hsp65 in leprosy patients: role of IL-18 and IL-13. *Clin Exp Immunol* 2004; 135(1):105-13.

Protection against intracellular pathogens such as

*Mycobacterium leprae* is critically dependent on the function of NK cells at early stages of the immune response and on Th1 cells at later stages. In the present report we evaluated the role of IL-18 and IL-13, two cytokines that can influence NK cell activity, in the generation of *M. leprae*-derived hsp65-cytotoxic T lymphocytes (CTL) from peripheral blood mononuclear cells (PBMC) of leprosy patients. We demonstrated that IL-18 modulates hsp65-induced CTL generation and collaborates with IL-12 for this effect. In paucibacillary (PB) patients and normal controls (N) depletion of NK cells reduces the cytolytic activity. Under these conditions, IL-12 cannot up-regulate this CTL generation, while, in contrast, IL-18 increases the cytotoxic activity both in the presence or absence of NK cells. IL-13 down-regulates the hsp65-induced CTL generation and counteracts the positive effect of IL-18. The negative effect of IL-13 is observed in the early stages of the response, suggesting that this cytokine affects IFN $\gamma$  production by NK cells. mRNA coding for IFN $\gamma$  is induced by IL-18 and reduced in the presence of IL-13, when PBMC from N or PB patients are stimulated with hsp65. Neutralization of IL-13 in PBMC from multibacillary (MB) leprosy patients induces the production of IFN $\gamma$  protein by lymphocytes. A modulatory role on the generation of hsp65 induced CTL is demonstrated for IL-18 and IL-13 and this effect takes place through the production of IFN $\gamma$ .

Hussain R, Shahid F, Zafar S, Dojki M, Dockrell HM. Immune profiling of leprosy and tuberculosis patients to 15-mer peptides of *Mycobacterium leprae* and *M. tuberculosis* GroES in a BCG vaccinated area: implications for development of vaccine and diagnostic reagents. *Immunology* 2004; 111(4):462-71.

*Mycobacterium leprae* (ML) GroES has been shown to induce strong T cell responses in tuberculoid as well as in exposed healthy contacts of leprosy patients, and therefore this antigen has been the focus of study as a potential vaccine candidate. Paradoxically, we have shown that ML GroES also induces extremely high titres of IgG1 antibody in leprosy patients across the disease spectrum, a response associated with disease progression. IgG1 antibodies in leprosy also show a negative association with interferon-gamma, a critical T cell cytokine responsible for macrophage activation and intracellular killing of mycobacteria. We therefore queried if antibody and T cell responses were being evoked by different epitopes in ML GroES proteins. To address the issue of epitope recognition in mycobacterial diseases, we have analysed 16 peptides (15-mer peptides) spanning the entire ML and *M. tuberculosis* GroES protein in leprosy (n = 19) and tuberculosis (n = 9) patients and healthy endemic controls (n = 8). Our

analysis demonstrates clearly that the dominant peptides evoking T cell and IgG subclass antibodies were different. The target of both T and B cell responses were cross-reactive epitopes in all groups. Differences in disease and healthy states related to the strength (mean intensity) of the T cell and antibody response. IgG1 and IgG3 antibodies were associated with disseminated disease and IgG 2 and IgG4 with disease limitation. Such comprehensive immune profiling of antigen-specific responses is critical to understanding the disease pathogenesis and also if these reagents are to be exploited for either diagnostic or vaccine purposes.

Pradhan V, Badakere SS, Shankar Kumar U. Increased incidence of cytoplasmic ANCA (cANCA) and other autoantibodies in leprosy patients from western India. *Lepr Rev* 2004; 75(1):50-6.

The prevalence of various autoantibodies was studied in 75 leprosy patients comprising eight patients with lepromatous leprosy (LL), 36 patients with borderline lepromatous leprosy (BL) and 31 patients with borderline tuberculoid leprosy (BT), along with 100 normal controls. Certain autoantibodies such as anti-nuclear antibodies (ANA), anti-single stranded DNA (anti-ssDNA) and anti-neutrophil cytoplasmic antibodies (ANCA) were raised among leprosy patients. When ANCA specificities to anti-myeloperoxidase (anti-MPO), anti-proteinase3 (anti-PR3) and anti-lactoferrin (anti-LF) were studied, it was found that the patterns of immunofluorescence such as perinuclear (p-ANCA), cytoplasmic (c-ANCA) and atypical (X-ANCA) and specificity by ELISA to anti-MPO, anti-PR3 and anti-LF varied in the LL, BL and BT groups. However, a higher amount of c-ANCA was observed in 62.5% of leprosy cases, while the incidences of p-ANCA and X-ANCA were lower. The LL group showed a higher incidence of autoantibodies as compared with the BL and BT groups, along with a male preponderance for autoantibody development. Some unusual antibody profiles such as 'X'-ANCA were also observed. The study suggests that autoantibody formation could be quite prevalent and also variable in the spectrum of leprosy cases, and there seems to be a serological overlap among leprosy and autoimmune disease, which could have pathogenetic importance in the leprosy patients developing complications.

Spencer JS et al. Comparative analysis of B- and T-cell epitopes of *Mycobacterium leprae* and *Mycobacterium tuberculosis* culture filtrate protein 10. *Infect Immun* 2004; 72(6):3161-70.

Culture filtrate protein 10 (CFP-10) from *Mycobacterium*

*tuberculosis* is a well-characterized immunodominant 10-kDa protein antigen known to elicit a very potent early gamma interferon response in T cells from *M. tuberculosis*-infected mice and humans. The sequence of the *Mycobacterium leprae* homologue of CFP-10 shows only 40% identity (60% homology) at the protein level with *M. tuberculosis* CFP-10 and thus has the potential for development as a T- or B-cell reactive antigen for specific diagnosis of leprosy. Antisera raised in mice or rabbits against recombinant *M. leprae* and *M. tuberculosis* CFP-10 proteins reacted only with homologous peptides from arrays of overlapping synthetic peptides, indicating that there was no detectable cross-reactivity at the antibody level. Sera from leprosy and tuberculosis patients were also specific for the homologous protein or peptides and showed distinct patterns of recognition for either *M. leprae* or *M. tuberculosis* CFP-10 peptides. At the cellular level, only 2 of 45 mouse T-cell hybridomas raised against either *M. leprae* or *M. tuberculosis* CFP-10 displayed a cross-reactive response against the N-terminal heterologous CFP-10 peptide, the region that exhibits the highest level of identity in the two proteins; however, the majority of peptide epitopes recognized by mouse T-cell hybridomas specific for each protein did not cross-react with heterologous peptides. Coupled with the human serology data, these results raise the possibility that peptides that could be used to differentiate infections caused by these two related microorganisms could be developed. Immunohistochemical staining of sections of *M. leprae*-infected nude mouse footpads resulted in strongly positive staining in macrophages and dendritic cells, as well as weaker staining in extracellular areas, suggesting that *M. leprae* CFP-10, like its homologue in *M. tuberculosis*, is a secreted protein.

Sridevi K, Khanna N, Chattree V, Pal PC; Haq W, Rao DN. Reversal of T cell anergy in leprosy patients: in vitro presentation with *Mycobacterium leprae* antigens using murabutide and Trat peptide in liposomal delivery. Int Immunopharmacol 2003; 3(12):1589-600

*Mycobacterium leprae*, the causative agent of leprosy resides and multiplies within the host monocytes and macrophages, thereby evading host immune system. Cell-mediated immune response (CMI) plays a vital role as evidenced from the high CMI in BT/TT (borderline and tuberculoid) patients and conversely low in BL/LL (borderline and lepromatous) patients. In the present study, an attempt was made to immunomodulate the anergized T cells of lepromatous leprosy patients by presenting the mycobacterial antigen in combination with T cell adjuvant, murabutide (active analog of muramyl' dipeptide, MDP-BE) and a Trat

peptide (T cell epitope of Integral membrane protein (Trat) from *Escherichia coli*) in particulate form (liposomes) or soluble form (media). PBMNC of normal, BT/TT and BL/LL were stimulated in vitro with five mycobacterial antigens (Ag) in the following formulations, Ag, Ag+murabutide, Ag+murabutide+Trat peptide either in liposomes or in medium. All the five antigen(s) when delivered in liposomes containing murabutide and Trate peptide showed a very high lymphoproliferative response ( $p < 0.001$ ) in all the three groups. IFN-gamma and IL-2 were significantly ( $p < 0.001$ ) high in these culture supernatants compared to IL-10 and IL-4 confirming a shift from CD4+Th2 to Th1 response in leprosy patients with particulate mode of antigen presentation. Interestingly, PBMNC derived from lepromatous patients also showed consistent T cell proliferation with all the formulations. Further, the mechanism of liposomal processing of antigens was studied using different inhibitors that interfere at different stages of antigen presentation. Results indicate that this study may pave way for an immunotherapeutic approach for reverting the anergic T cells of lepromatous patients to proliferating T cells with the release of Th1 cytokines thereby restoring the CMI response in these patients.

## Imunoterapia

Stanford J, Stanford C, Grange J. Immunotherapy with *Mycobacterium vaccae* in the treatment of tuberculosis. Front Biosci 2004; 9:1701-19.

All the trials of immunotherapy of tuberculosis with killed *Mycobacterium vaccae*, published or not, that are known to the authors are reviewed here. Following an introduction giving a brief account of some earlier immunotherapies for tuberculosis, the origins of the concept of immunotherapy with *M. vaccae* are considered. Progress is traced from the early work with irradiation-killed organisms in leprosy to the study in London of modulation of tuberculin skin-test responses, and the first comparative trials in The Gambia and Kuwait. In the last of these studies, dosages and different preparations were compared. As a result of this subsequent studies have used 109 heat-killed organisms, equivalent to 1mg wet-weight of bacilli, as a standard dose. A series of small trials in Argentina, India, Nigeria, Romania, South Africa and Vietnam have pioneered the way forward, disclosing geographic variability, with South Africa as the only country where almost no effects were recorded. Together the studies have shown that a single dose may not be sufficient. These studies have confirmed the mode of action of *M. vaccae* to be regulation of cell-mediated immunity with enhancement of Th1 and down-regulation of Th2, and they have shown benefits in faster bacteriological conversion,



reduction in ESR, recovery of body weight and resolution of radiological opacities, leading to better recovery from the disease even when given to patients receiving directly observed therapy, short-course (DOTS). Three major randomised, placebo-controlled and partly blinded trials have been carried out in Africa. The first, in South Africa showed no *M.vaccae*-related effects. The second trial, in Uganda, confirmed the observations made in the earlier studies of faster sputum conversion and better radiological clearance. The third trial, in Zambia and Malawi, showed a trend towards benefits in the treatment of HIV seronegative patients but failed to show beneficial effects in HIV seropositive patients. Studies in patients with multi-drug-resistant tuberculosis have shown that multiple doses of immunotherapy are required in most cases, and that these markedly improve cure-rates for these patients. This is especially so when they are also treated with chemotherapy tailored to the resistance pattern of their infecting organisms. A small study has just commenced in which repeated doses of *M.vaccae* are being administered to a group of patients who have failed treatment with DOTS-Plus (directly observed therapy with drugs selected on the basis of drug susceptibility profiles). Late in the investigation came publications from China supporting and confirming the data in both drug-sensitive and drug-resistant disease, by the use of multiple injections of their own different preparation of *M.vaccae*. The trial that is now beginning in Vietnam of 3 doses of *M.vaccae* in the treatment of newly diagnosed pulmonary tuberculosis, is accompanied by a chemotherapeutic regimen with a shortened continuation phase. If this important study is successful, immunotherapy with killed *M.vaccae* should be introduced into the treatment regimens for tuberculosis worldwide.

### Infecção Experimental

Job CK. Nine-banded armadillo and leprosy research. *Indian J Pathol Microbio* 2003; 46(4):541-50.

In this presentation an attempt has been made to describe the nine-banded armadillo as an animal model, probably the only one in which lepromatous leprosy similar to that found in humans can be experimentally produced. Some unique features of the physiology of the animal are mentioned. The pathology and the microbiology of leprosy in the armadillo are described in detail. The discovery of lepromatous leprosy in the wild armadillos in the southern parts of United States, the transmission of disease among them through trauma and thorn pricks and the pathogenesis of the disease are presented. The impact of leprosy in the wild animals may have on human leprosy is discussed.

### Neurologia

Grover C, Kubba S, Nanda S, Kumar V, Reddy BS. Leprosy with Guillain Barre Syndrome: a new neurologic manifestation? *J Dermatol* 2004; 31(2):119-23.

A 19-year-old female patient of lepromatous leprosy with Type II reaction, on multidrug therapy and prednisolone, presented with acute onset flaccid quadriplegia. The cerebrospinal fluid examination revealed albumino-cytologic dissociation. Nerve biopsy showed infiltration with lepra bacilli, features of vasculitis, and demyelination. There were no other identifiable precipitating factors for Guillain Barre Syndrome in this patient. Her condition improved without any steroid therapy. This case emphasizes the hypothesis that cell injury caused by Type II reaction can expose neural antigens and incite an autoimmune reaction in the form of Guillain Barre Syndrome.

Haanpää M, Lockwood DN, Hietaharju A. Neuropathic pain in leprosy. *Lepr Rev* 2004; 75(1):7-18.

Chronic neuropathic pain in treated leprosy has received scant attention. In this article the concept, clinical features and diagnosis of neuropathic pain are reviewed. The possible pathophysiological mechanisms, treatment challenges and research needs in this area are discussed.

Schön T, Hernández-Pando R, Baquera-Heredia J, Negesse Y, Becerril-Villanueva LE, Eon-Contreras JC et al. Nitrotyrosine localization to dermal nerves in borderline leprosy. *Br J Dermatol* 2004; 150(3):570-4.

Background: Nerve damage is a common and disabling feature of leprosy, with unclear aetiology. It has been reported that the peroxidizing agents of myelin lipids-nitric oxide (NO) and peroxynitrite-are produced in leprosy skin lesions. Objectives: To investigate the localization of nitrotyrosine (NT)-a local end-product of peroxynitrite-in leprosy lesions where dermal nerves are affected by a granulomatous reaction. Methods: We investigated by immunohistochemistry and immunoelectron microscopy the localization of the inducible NO synthase (iNOS) and NT in biopsies exhibiting dermal nerves from patients with untreated leprosy. RESULTS: There were abundant NT-positive and iNOS-positive macrophages in the borderline leprosy granulomas infiltrating peripheral nerves identified by light microscopy, S-100 and neurofilament immunostaining. Immunoelectron microscopy showed NT reactivity in neurofilament aggregates and in the

cell wall of *Mycobacterium leprae*. Conclusions: Our results suggest that NO and peroxy-nitrite could be involved in the nerve damage following borderline leprosy.

## Odontologia

Núñez-Martí JM, Bagán JV, Scully C, Peñarrocha M. Leprosy: dental and periodontal status of the anterior maxilla in 76 patients. *Oral Dis* 2004; 10(1):19-21.

Objective: To evaluate the presence of oral disease, as assessed by dental and periodontal indices, in the anterior maxilla of a group of 76 patients with leprosy, compared with a group of matched control subjects. Materials and Methods: The study included 76 patients with leprosy (age range 40-82 years; 39 males), resident in the sanatorium of San Francisco de Borja de Fontilles (Alicante, Spain). Clinical examination was carried out to evaluate the decayed missing and filled index, and the periodontal status in the anterior maxilla, using the Löe-Silness dental plaque index, mean periodontal probing depth and the average periodontal attachment loss. RESULTS: In the leprosy patients, a large proportion of maxillary incisors and canines were missing. The mean plaque index in leprosy was 2.35 +/- 0.7, with a probing depth of 2.96 +/- 0.8, and an average attachment loss of 4.18 +/- 1.3, indices all statistically greater than in controls. There were no differences detected in the oral indices measured according to the presence or absence of facial destruction or the type of leprosy. Conclusions: Patients with leprosy show a tendency to poor dental and periodontal health, unrelated to the presence of facial destruction or the type of leprosy.

## Oftalmologia

Kondo S. [Ocular impairment in leprosy]. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):23-35.

Leprosy causes several ocular disorders, and it also causes aftereffect with high frequency in various ways. Primary impairment is the ocular disturbance caused with direct invasion of nerve and ocular tissue by *Mycobacterium leprae*. Secondary impairment is the complication of nerve paralysis and residual inflammation due to primary disorder. Main work at Japanese national leprosariums has been the control of primary and secondary impairment in recent years. Clinical ophthalmic study in the leprosarium revealed an increase of age-related ocular disease in addition to aftereffect of leprosy. Severe sequelae due to sensory and functional disturbance will require suitable applications of advanced clinical technologies.

Nepal BP, Shrestha UD. Ocular findings in leprosy patients in Nepal in the era of multidrug therapy. *Am J Ophthalmol* 2004; 137(5):888-92.

Purpose: Detailed ophthalmic evaluation was performed to determine the prevalence of ocular complications among leprosy patients on multidrug therapy and those released from multidrug treatment. Design: Observational case series. Methods: Leprosy patients at Tribhuvan University Teaching Hospital from April 1, 2001, through September 30, 2002, underwent detailed ophthalmic evaluation including slit-lamp biomicroscopy, dilated funduscopy, and applanation tonometry. Results: We evaluated 58 leprosy patients. A majority (72%) was receiving treatment for multibacillary leprosy; 14% belonged to posttreatment multibacillary and paucibacillary groups. Ocular involvement was found in 57% of patients. In the multibacillary group, 55% had ocular involvement, which was more than double that found in the paucibacillary group (25%), although this finding was not statistically significant ( $P = .187$ ). Among patients with ocular complications, 48% had visual disability and another 45% had threatened vision; 9% met World Health Organization guidelines for blindness. Uveitis and its complications were the predominant causes of visual disability (88%). Conclusion: Ocular complications and visual disability are high among leprosy patients in Nepal even after completing multidrug therapy.

## Reabilitação

Yan L, Zhang G, Zheng Z, Li W, Zheng T, Watson JM, Piefer A. Comprehensive treatment of complicated plantar ulcers in leprosy. *Chin Med J (Engl)* 2003; 116(12):1946-8.

Objective: To investigate feasible treatment methods for plantar ulcers in leprosy patients according to the agreement between the Ministry of Health (MOH) of China and the Leprosy Mission International (LMI). Methods: A total of 2599 complicated foot ulcers in 1804 leprosy cases underwent surgical treatment. Plastic fixation and supports were used, dressings were changed regularly, and protective footwear and modified insoles were provided. Results: Of the 2599 foot ulcers 1446 (55.64%) healed. The cure rate of the patients treated in leprosy hospitals was 71.31%, with 219 (15.15%) recurrences of foot ulcers. The recurrence rate of those who lived at home was 18.35%. Conclusions: Comprehensive treatment of foot ulcers has a high cure rate and a low recurrence rate. Reduction of workload, avoidance of long distance walking, intensification of education on foot self-care and provision of financial support are the main measures for preventing a recurrence of foot ulcers.

## Terapêutica

Gidoh M, Namisato M, Kumano K, Goto M, Nogami R, Ozaki M. [Guideline for the treatment of leprosy by new quinolones]. *Nihon Hansenbyo Gakkai Zasshi* 2004; 73(1):65-7.

Ofloxacin(OFLX) is often applied today as a substitution drug of MDT for drug resistance to dapsona, rifampicin or

clofazimine. However, OFLX resistance is also becoming a great concern. Low and/or irregular administration are considered to be the major causes of OFLX resistance. OFLX should be used as a combined therapy, and minimal daily dose of 400 mg of OFLX or 200-300 mg of levofloxacin is required. Quinolone resistance should be considered when no improvement of clinical and/or bacterial index is observed after the treatment for 6 months. In such cases, resistance gene detection is necessary.