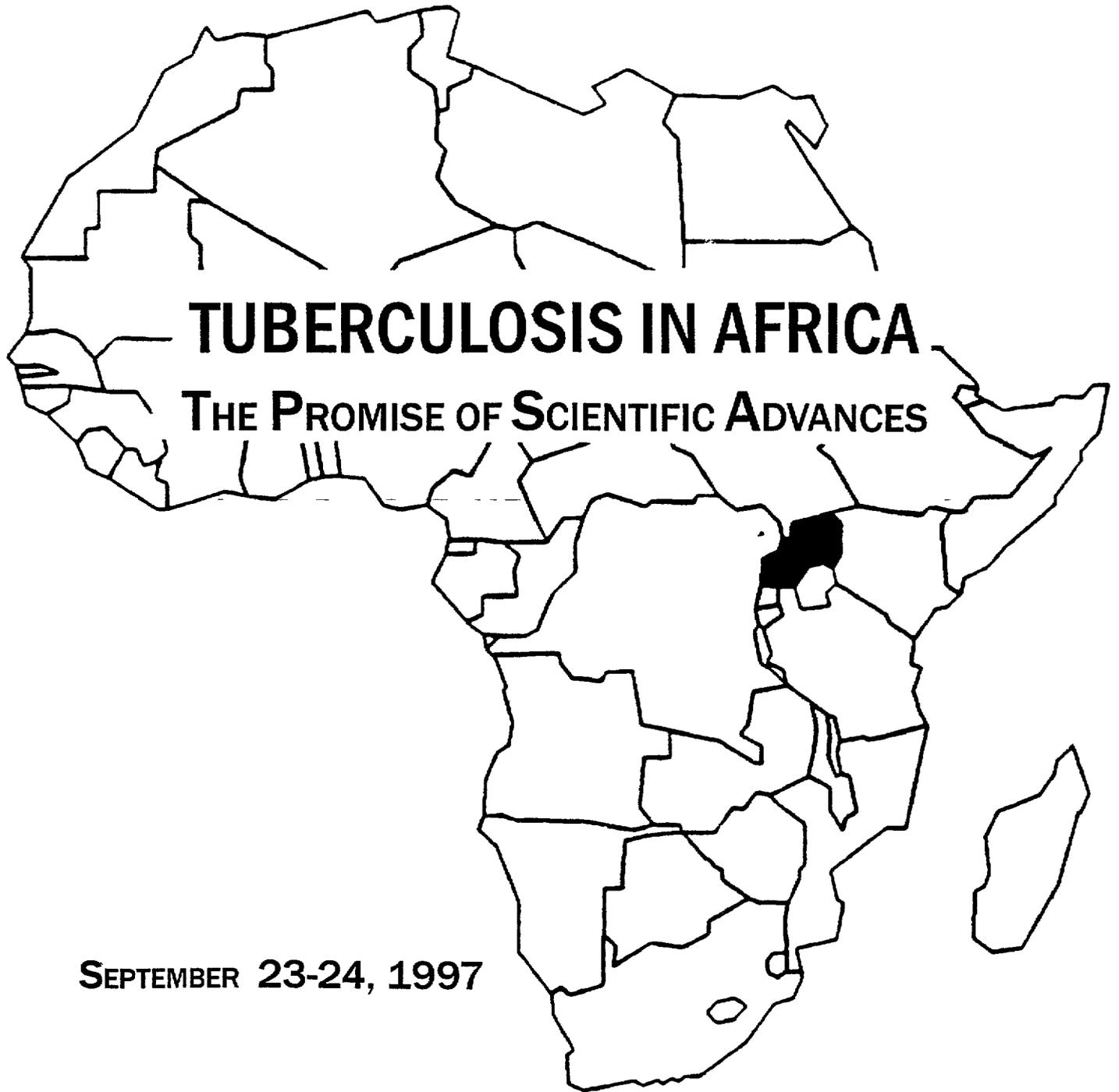


PROCEEDINGS FROM



TUBERCULOSIS IN AFRICA

THE PROMISE OF SCIENTIFIC ADVANCES

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TABLE OF CONTENTS

The History of the Uganda-CWRU Research Collaboration (US perspective)	1
<i>Thomas Daniel</i> Case Western Reserve University	
The History of the Uganda-CWRU Research Collaboration (Uganda perspective)	1
<i>Sam Okware</i> Ugandan Ministry of Health	
The Importance of Scientific Research for Tuberculosis Control in Africa	2
<i>Jerrold Ellner</i> Case Western Reserve University	
Tuberculosis in Africa - The Current Situation	3
<i>Dermot Maher</i> WHO Global Tuberculosis Programme	
HIV - TB Interactions and their Relevance to Uganda	4
<i>Christopher Whalen</i> Case Western Reserve University	
Transmission of Tuberculosis in Ugandan Households	6
<i>David Guwatudde</i> Makerere University	
Challenges in Tracing Tuberculosis Contacts - The Ugandan Experience	7
<i>Margaret Nakakeeto</i> Makerere University	
Tuberculosis in Ugandan Children	8
<i>Philippa Musoke-Mudido</i> Makerere University	
The Impact of HIV on Transmission of Tuberculosis	8
<i>David Guwatudde</i> Makerere University	
Molecular Epidemiology of Tuberculosis in Uganda	9
<i>Moses Joloba</i> Makerere University	
Cytokine Regulation of the Host Response to <i>M. tuberculosis</i> infection	9
<i>Gilla Kaplan</i> Rockefeller University	
Cytokine/Chemokine Cascades in the Immunopathology of Tuberculosis	10
<i>Ian Orme</i> Colorado State University	

Cytokine Profiles in Tuberculosis <i>Catherine Othienio/Christina Hirsch</i> <i>Makerere University/Case Western Reserve University</i>	10
Immune Activation, and Mortality in HIV-Positive Tuberculosis Predictors of Outcome and Strategies for Intervention <i>Robert Wallis</i> <i>Case Western Reserve University</i>	11
Surrogate Markers and Response to Treatment of Tuberculosis <i>Moses Joloba</i> <i>Makerere University</i>	13
Current Status of Vaccination for the Prevention of Tuberculosis <i>Paul Fine</i> <i>London School of Hygiene and Tropical Medicine</i>	13
Development and Testing of New Tuberculosis Vaccines <i>Douglas Young</i> <i>Imperial College of Medicine at St Mary's Hospital</i>	14
Novel Strategies for TB Control Finding Gold in Soil and Cow Dung <i>William Jacobs</i> <i>Albert Einstein University</i>	14
BCG Case-Control Study in HIV-infected Women <i>Francis Engwau-Adatu</i> <i>Uganda National TB and Leprosy Control Programme</i>	15
<i>Mycobacterium vaccae</i> - The South African Experience <i>Bernard Fourie</i> <i>Medical Research Council - South Africa</i>	15
<i>Mycobacterium vaccae</i> - The Ugandan Experience <i>Moses Kamva</i> <i>Makerere University</i>	15
Can New Rapid TB Diagnostics be Applied in Africa <i>Kathleen Eisenach</i> <i>University of Arkansas</i>	17
Advances in the Treatment and Prevention of Tuberculosis <i>Richard O'Brien</i> <i>Centers for Disease Control and Prevention</i>	19
Rifampicin and Thiacetazone Treatment Regimens in HIV-infected Tuberculosis Patients <i>Alphonse Okwera</i> <i>Uganda National TB and Leprosy Control Programme</i>	19

	3
Role of Ethambutol in the Consolidation Phase of Tuberculosis Treatment Regimens	20
<i>Henry Luzze</i> <i>Makerere University</i>	
Mycobacterial Drug Resistance in Uganda	21
<i>Thomas Aisu</i> <i>Makerere University</i>	
Relapses in HIV-infected Adults with Tuberculosis	22
<i>John L Johnson</i> <i>Case Western Reserve University</i>	
Preventive Treatment of Tuberculosis in HIV-infected Adults	23
<i>Roy Mugerwa</i> <i>Makerere University</i>	
Notes	25

The History of the Uganda-CWRU Research Collaboration (U S perspective)

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In 1987, a group of Case Western Reserve University (CWRU) faculty members affiliated with the newly formed CWRU Center for International Health responded to an invitation from the United States National Institutes of Health (NIH) to submit proposals to establish International Centers for AIDS Research (ICAR). With receipt of an award to establish such a Center at Makerere University for the purpose of conducting studies of (1) Mother-Child Vertical Transmission of AIDS, (2) Tuberculosis and AIDS, (3) Social Impact of AIDS, and (4) AIDS-Associated Neoplasms, the Uganda-CWRU collaboration was born. The CWRU Principal Investigator was Frederick C. Robbins, his Ugandan counterpart Sam Okware.

Recognition that the ICAR funds would be of short duration and of limited amount led CWRU investigators to look to other agencies for funds and to expand the scope of the collaboration to include other related scientific investigations. A pediatric AIDS clinic was established, and in it studies were conducted not only of vertical transmission, but also of neurodevelopment in HIV-infected children and of the efficacy of HIV-specific human immunoglobulin in prevention of neonatal HIV infection. Grants were obtained from Rotary International to provide nutritional support to children enrolled in these studies.

Additional funds were obtained to support studies of tuberculosis and AIDS, most notably the large award to CWRU by the NIH in 1994 to establish the Tuberculosis Research Unit (TBRU) directed by Jerrold J. Ellner. Collaborative studies of the impact of AIDS upon the presentation and course of tuberculosis, the serologic and microbiologic diagnosis of tuberculosis in HIV-infected persons, the epidemiology and transmission of tuberculous infection, and the treatment and prophylaxis of tuberculosis in HIV-infected individuals have been conducted. With a shift in emphasis at the NIH, the original ICAR gave way to cohort studies undertaken in Preparation for AIDS Vaccine Evaluation (PAVE). The new Ugandan Joint Clinical Research Centre joined

the collaboration to participate in these and other studies.

In parallel with the research collaboration, joint educational ventures were undertaken. With Rockefeller Foundation support, a new baccalaureate nursing curriculum for Uganda was developed. The NIH Fogarty International Center provided funds that have been used to support post-doctoral degree studies in Epidemiology and Biostatistics for Ugandans at CWRU. The University Development Linkages Program of the U.S. Agency for International Development funded a linkage that supported the redevelopment of the Makerere University Institute for Public Health.

The History of the Uganda-CWRU Research Collaboration (Uganda perspective)

*Sam Okware
Ugandan Ministry of Health*

As far back as the late 1980's, Uganda realized the need for scientific solutions to the HIV/AIDS epidemic that had emerged as a serious threat to the country. Collaborative relationships were established with international organizations and institutions, including Case Western Reserve University, Columbia University, University of California at San Francisco, and others to provide Uganda with the necessary expertise and technology to enable the country to quickly tackle the problem at hand.

The formal collaboration between CWRU, Cleveland, Ohio, Makerere University, and the Ministry of Health began in August 1988 through a program supported by an International Collaboration for AIDS Research (ICAR) grant from the US National Institutes of Health. In addition, the Fogarty International Center (NIH) funded and has renewed a five-year AIDS International Training and Research (AITRP) training grant to CWRU to support degree and non-degree training of Ugandans in the US and Uganda since 1989. Additional educational assistance is provided through support to the Makerere Medical School's Albert Cook Library.

About 30 graduates have completed the Master of Science and Ph D training in public health epidemiology, microbiology and anthropology, and now serve in important positions within the Ministry of Health, the Medical School, the Institute of Public Health, and the National Tuberculosis and Leprosy Control Programme. Numerous courses in diverse areas, such as computer data base management, management of adult and pediatric HIV/AIDS, AIDS vaccine development, research methodology, pulmonary disease and TB, and sexually transmitted diseases have benefited medical students, nurses, laboratory technologists, post-graduates in training at Mulago Hospital and other members of the University and Hospital communities.

In October 1994, CWRU was awarded a major contract as a US National Institutes of Health-sponsored TB Prevention and Treatment Research Unit (TBRU). The Uganda-CWRU Research Collaboration TB Project clinic is one of the principal sites for epidemiology, developmental microbiology, immunology and phase I/II clinical trials.

Currently, multidisciplinary research areas of the Collaboration include tuberculosis and HIV interactions, TB preventive therapy in HIV-infected individuals, TB transmission and immunology, preparation for AIDS vaccine field studies, a phase I trial of a potential HIV vaccine (vCP205 - recombinant canarypox vector vaccine), and behavioral research. These are funded through the US National Institutes of Health, the US Centers for Disease Control and Prevention, the World Health Organisation, pharmaceutical companies and private benefactors.

The research has led to over 50 peer-reviewed publications and about 150 abstracts and presentations at national and international meetings.

As a result of the collaboration our infrastructure has been developed. An area of Old Mulago Hospital, near the Uganda Cancer Institute, was extensively renovated beginning in 1988 to provide space for central administrative offices, a computerized data management area, TB project

offices and clinic examination rooms shared by ongoing research projects of the Collaboration. A two-story building sponsored through USAID provides state-of-the-art core laboratory and conference room facilities. Additional infrastructure includes a developmental mycobacteriology laboratory at the Joint Clinical Research Centre, has been developed in Kampala to support the clinical trials, and microbiology component of the TB research unit.

Investigators of the Uganda-CWRU Research Collaboration have worked closely with colleagues in the Ugandan AIDS/STD Control Programme, the Institute of Public Health, Makerere University Medical School, the Uganda Virus Research Institute in Entebbe, the Ugandan AIDS Commission, the Ugandan Ministry of Health, and the Ugandan National Tuberculosis and Leprosy Programme. This cooperation has contributed to better patient investigation and management.

The Importance of Scientific Research for Tuberculosis Control in Africa

Jerrold Ellner

Case Western Reserve University

The grim projections for TB morbidity and TB mortality as we enter the next millennium indicate that TB control efforts have failed. The burden to developing countries is disproportionate. Over 90% of new cases and 95% of deaths occur in developing countries. An active debate has raged for too long as to whether we need better tools to control TB or better application of existing tools. Quite clearly, both are necessary. The application of new tools also becomes a reasonable option in an era of resurgence of interest in basic research on tuberculosis and marked by the sequencing of the *M. tuberculosis* genome.

The current approaches to prevent, diagnose and treat TB are badly outmoded. For example, the current vaccine, BCG was developed over 70 years ago and is based on a spontaneous mutation of *Mycobacterium bovis*. It only appears to be effective in one-half of cases. Can

we do better at a time that molecular genetics allows mutations with incredible precision? Auxotrophic libraries of less virulent *M tuberculosis* and of BCG are being developed Individual protein, such as those of the 85B antigen complex, have been protective as subunit vaccines in animal models, as have DNA vaccines containing the relevant genes Further development of TB vaccines now is in the hands of manufacturers The prospects for human trials are high It is less certain whether existing animal models and human immunologic surrogates for protective immunity can be applied to select the best candidates before committing to large trials of vaccine efficacy In addition to the potential use of vaccines for primary prevention of *M tuberculosis* infection, vaccine trials are being considered for secondary prevention—that is administration to the already-infected to prevent disease

Scientific progress also has brought us to clinical trials of immunotherapy in patients with TB Two controlled trials of heat-killed *M vaccae* as an adjunct to chemotherapy of pulmonary TB are nearing completion and promising data have emerged concerning human recombinant interleukin 2 Immunotherapy may prove a useful adjunct to standard drug therapy and also could activate the immune response to destroy persisting organisms and permit a shorter course of therapy in drug sensitive TB

Sensitive and highly specific molecular diagnostics such as polymerase chain reaction have been applied successfully to the diagnosis of TB They are "high-tech" and expensive There has been a resurgence of interest in the development of inexpensive diagnostics for application in resource poor countries Progress has been made recently in serodiagnosis and in antigen detection

The last drug developed for the treatment of TB, rifampin was approved 30 years ago Two more potent rifamycins with longer half-lives, rifapentine and KRM1648 are undergoing clinical trial Large programs exist for screening available drugs for anti-tuberculous activity in vitro and in animal models Even more ambitious attempts to develop new drugs are based on recognizing new

potential targets as the biochemistry of the organism is elucidated

Molecular epidemiology based on DNA fingerprinting has and will clarify the current epidemiology and transmission of TB thereby examining the basic premises of TB Control programs

Nonetheless, scientific advances of the past may seem paltry in light of the quantum leap forward expected to ensue from sequencing of the TB genome Virulence factors, vaccine candidates, and new drug targets should become apparent in a fairly rapid fashion

Currently, it can be pointed out that science has not contributed to TB Control in the last 30 years Quite clearly, this will change dramatically in the next decade It is even possible to dream that TB will join the list of potentially "eradicateable" infectious diseases

Tuberculosis in Africa - the current situation

Dermot Maher

WHO Global Tuberculosis Programme

The global TB burden is increasing because of a) poverty and the widening gap between rich and poor in various populations, b) neglect of TB control (inadequate case detection, diagnosis and cure), c) changing demography (increasing world population and changing age structure), d) the impact of the HIV pandemic Ninety-five percent of estimated TB cases and 98% of estimated TB deaths occur in developing countries These deaths comprise 25% of all avoidable deaths in developing countries Eighty percent of cases are in the economically productive age group (15-59 years) After years of declining incidence, the number of reported cases of TB has increased dramatically since the 1980's in many countries of sub-Saharan Africa The increased number of cases poses a challenge to health services, TB control programmes, and clinicians The role of researchers is to help these to meet the challenge

Of the 3.25 million TB cases notified world-wide in 1995, 0.45 million (14%) were in the African

Region Case notifications represent only a fraction of true incident cases. With an estimated total number of new TB cases in 1995 of 1.25 million, sub-Saharan Africa is the region with the highest incidence of TB. WHO estimated that world-wide about 22 million people were living with HIV infection in mid-1996, with 14 million (64%) in sub-Saharan Africa. World-wide there were 9.4 million persons with HIV and TB co-infection, of whom the great majority were in sub-Saharan Africa (6.6 million). In 1995, the estimated proportion of TB cases attributable to HIV infection was 9% world-wide, but 26% in sub-Saharan Africa. In many Eastern and Southern African countries HIV seroprevalence rates in new TB patients are 30-60 percent.

WHO has used "DOTS" as a "brand name" for the recommended TB control strategy. The recently established WHO global TB surveillance and monitoring project has provided information on the status of implementation of the WHO recommended DOTS strategy by reporting countries. Of the 216 countries and territories surveyed by WHO in 1995, 70 have adopted the recommended strategy. Out of the 48 countries in the Africa region, 29 have accepted the strategy, of which 16 have country-wide implementation. Drug-resistant, and particularly multi-drug resistant, TB poses a serious challenge to global control, especially in the face of the HIV epidemic.

The TB burden in Africa will continue to increase without effective TB control programs. Good TB control costs a certain minimum amount of money. In many developing countries, government per capita expenditure on health is falling, and expenditure on national TB control programs is often not protected. The political will necessary for global TB control must translate into the commitment of governments to provide adequate funds to implement effective programs that can be sustained for several decades. The mobilization of this political will constitutes a huge challenge for all concerned with TB control. Researchers have the responsibility to address the issues relevant to helping national TB programs achieve high cure rate and case-finding targets.

HIV – TB Interactions and their Relevance in Uganda

Christopher Whalen
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The World Health Organization estimated that by 1995, 5.6 million individuals worldwide were dually infected with HIV-1 and *M. tuberculosis*. Since both organisms are intracellular parasites and induce cell mediated immune responses, it is reasonable to postulate a bi-directional relationship between HIV-1 and *M. tuberculosis*. The hallmark of HIV-1 infection is the progressive decline and dysfunction of CD4+ lymphocytes and the resulting immunodeficiency. HIV-1 infection confers the greatest known risk for the development of active tuberculosis, both for reactivation of latent infection or progressive primary disease. It alters the clinical presentation of tuberculosis and has been associated with outbreaks of multi-drug resistant organisms. Early in the HIV epidemic, researchers postulated that the immune activation resulting from concurrent infection with parasitic or bacterial pathogens might alter the natural history of HIV infection. In a retrospective cohort study of HIV-infected women in Zaire, women with tuberculosis were about 3 times more likely to die relative to women without tuberculosis. Similar unpublished observations from Uganda, led to a theory of co-pathogenesis of TB and HIV-1.

This theory states that the immune response to tuberculosis is associated with activation of macrophage and lymphocytes. The activation of these cells is dependent upon mycobacterial products and is mediated by cytokines. This cellular activation enhances HIV expression and may lead to increased viral load, reduced in CD4+ lymphocyte counts and increased risk for opportunistic infections. The concurrence of these diseases leads to a vicious cycle of immunosuppression and amplification of HIV load.

Studies of the immune response in TB provide insights and biologic plausibility for co-pathogenesis in dually-infected persons. The initial interaction between the host immune system and *M. tuberculosis* occurs in the alveoli. Pulmonary macrophage engulf, process and present mycobacterial antigens in conjunction

with MHC class II molecules. When this complex is recognized by CD4+ cells, they release interferon- γ (INF- γ) and IL-2. The INF- γ serves to activate macrophages and enhance their ability to kill microorganisms. The activated macrophages release a number of cytokines including TNF- α , IL-1, and IL-6 which in turn activate the CD4+ cell. This process is down-regulated by the macrophage-derived TGF- β and IL-10. Within this panoply of cytokines, TNF- α deserves emphasis because it is so closely aligned to TB pathogenesis. TNF- α is essential for granuloma formation and activates macrophage clearance of the organism. Increased levels of TNF α are found in the serum and pleural fluid of TB patients. Further, *Mycobacterium tuberculosis* and its polysaccharide (lipoarabinomannan) and protein (58 kD glutamate synthetase, 30 kD alpha antigen mycolyl transferase) products directly stimulate mononuclear phagocytes to express TNF α as well as other cytokines. TNF- α also appears responsible for many of the systemic signs and morbidity of TB such as fever, sweats and cachexia.

There is mounting evidence in favor of the co-pathogenesis theory of TB and HIV-1. Mycobacteria and mycobacterial products convert latent HIV infection of macrophage cell lines to a productive infection through a mechanism that depends, at least in part, upon the induction of TNF α expression. CD4+ lymphocytes from TB patients show increased expression of Class II MHC and their monocytes increased surface Fc γ RI and Fc γ RIII, in each case, activation is more pronounced in the dually-infected TB/HIV patients. This also is true for the serum markers of activation, neopterin and β 2-microglobulin as well as serum TNF- α . Further, the level of activation of monocytes in TB is sufficient to enhance susceptibility to productive infection with HIV *in vitro*. TNF- α expression by PPD-stimulated PBMC is higher in TB/HIV than TB alone or HIV alone. The most recent supportive data derive from quantification of viral load in patients with HIV-associated TB. In a group of cases and controls matched for CD4+ count, age and sex, TB/HIV was associated with approximately 5-fold higher levels of plasma HIV RNA in the cases than controls. Further, two published

studies have now shown that active TB modulates the expression of HIV as measured by HIV RNA copies both in peripheral blood mononuclear cells and pulmonary macrophage. Moreover, treatment of TB led to a reduction in subsequent viral load levels. From cohort studies in Uganda, additional data indicate that the development of incident TB in HIV-infected individuals is associated with a rise in plasma viral load and a concurrent decline in CD4+ cells. Studies have shown that both cytokine and mycobacterial products up-regulate HIV expression in chronically-infected T- and macrophage cell lines by inducing transcription activating factors that bind to the NF κ B binding site in the HIV-LTR.

HIV-1 and *M. tuberculosis* also interact at the clinical and population level. HIV-1 infection increases the mortality associated with active tuberculosis despite adequate response to therapy, the one-year mortality from TB in HIV-infected individuals is over 4 times the rate in HIV seronegative cases. In the US, the HIV-1 epidemic has also increased the number and rate of deaths due to tuberculosis, especially among younger adults. Whereas these studies emphasize the impact of HIV-1 on the natural history of tuberculosis, the effect of dual infection may be bi-directional. Epidemiologic data indicate that active tuberculosis may affect the clinical course of HIV infection. In a retrospective cohort study of HIV-infected women from Zaire, the relative risk of death was estimated to be 2.7 in those with active tuberculosis compared to those without tuberculosis despite presumably appropriate anti-tuberculous therapy. In a retrospective cohort study of HIV-infected subjects done in the US, active tuberculosis was associated with an increased risk for death (OR = 2.2) and an increased incidence of opportunistic infections, even when controlling for age, intravenous drug use, previous opportunistic infection, baseline CD4+ count, and anti-retroviral therapy. The risk of death, or hazard rate, in HIV-associated tuberculosis follows a bimodal distribution, peaking within the first three months of therapy, and then again after one year, the reasons for this bimodal distribution are not clear but may relate to the impact of TB on HIV disease progression. The observation that active tuberculosis increases mortality associated with HIV infection has now been corroborated in

a number of independent cohorts in Europe and Africa. In a prospective cohort study from Uganda, the survival distributions of HIV-infected individuals with and without TB have been compared. When adjusting for CD4+ count, degree of immune activation as measured by the b2 microglobulin, previous HIV-related morbidity, and demographic factors, the relative risk of death in TB patients versus the controls without TB was 1.64 ($P = 0.02$). The effect of TB on survival was most pronounced in subjects with less advanced immunosuppression and survival was associated with the degree of immune activation.

These results are consistent with the hypothesis that tuberculosis acts as a co-factor in disease progression of HIV, although other explanations are plausible. The major competing hypothesis is that active tuberculosis is merely a marker for more severe immunosuppression at a given level of absolute CD4+ lymphocyte count.

In summary, HIV-associated TB is characterized by activation of macrophage and CD4+ lymphocytes and mediated by pro-inflammatory cytokines. HIV expression is enhanced by these cytokines and mycobacterial products leading to increased viral load and reduction in CD4+ lymphocyte count. In HIV-infected persons, TB is associated with an increased risk for death, especially in individuals with preserved immune function.

Transmission of Tuberculosis in Ugandan Households

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Objectives 1) Determine prevalence of TB infection and disease in Ugandan households with a recently identified sputum smear-positive adult with TB compared to those without previously known TB cases, 2) Assess household versus community transmission of tuberculosis in Ugandan households.

Design A neighborhood-matched, cross-sectional prevalence study of active TB and tuberculin sensitivity using the Mantoux technique (5 TU) within households.

Setting & Participants Index cases with sputum smear-positive tuberculosis, living within a radius of 20 km from Kampala city center, were identified and randomly selected at the National TB Treatment center at Old Mulago Hospital, the largest TB clinic in Kampala District, Uganda. All full-time household members (contacts) of the index case were eligible for inclusion in this study. Control households were selected at random from the neighboring community, and household members were similarly evaluated.

Measurements In addition to assessing demographic and household environment, all household members had a clinical evaluation which included medical history & physical examination, a PPD tuberculin skin-test (read within 48-72 hours), BCG vaccination status, HIV test (among consenting subjects) and presence of any TB symptoms. Any household member with signs of TB underwent a complete TB work-up. If active TB was diagnosed, the subject was put on standard TB chemotherapy. Measurements were made using standardized interview and review by an independent panel of physicians.

Main Outcome Measures 1) Tuberculin skin-test reactivity (skin-test reading ≥ 5 mm was considered positive), 2) Active TB.

Results 147 index TB cases and their household contacts, and 147 control households are included in this analysis. Of the 592 contacts in TB case households, 80% were PPD-positive and 6% (38 cases) had active TB. The majority of subjects with active TB were children aged ≤ 5 years (29 cases). Active TB was culture confirmed in all contact TB cases aged > 5 years, and in 34% of subjects aged ≤ 5 years. Of the 805 subjects in control households, 46% were PPD-positive and culture-confirmed TB cases were identified in five eligible control households of which two were children aged ≤ 5 years. The adjusted Odds Ratio of PPD positivity in contacts vs non-contacts was 5.88 [95% CI 4.35-7.69], while the Community Infection Ratio (CIR) was 0.17 [95% CI 0.13-0.23]. PPD positivity was significantly higher in older subjects, adjusted odds ratio = 1.03 [95% CI 1.03-1.04]. PPD reactivity was higher in BCG vaccinated infants compared to unvaccinated infants, however, this difference did not attain statistical significance.

No significant associations were noted between PPD-positivity and household size, sex and nutritional status

Conclusion The prevalence of infection with *M tuberculosis* in Uganda is high, and transmission rates are high in households of sputum smear-positive adults. Household contacts of persons with smear-positive TB are at increased risk for developing TB. This risk is higher in children less than five years.

¹The Community Infection Ratio (CIR) is a measure of the relative importance of community versus household transmission. Madico G, et al, *Lancet* 1995, 345: 416-19

$CIR = \frac{[Prevalence\ in\ Non-contacts / (1 - Prevalence\ in\ Non\ contacts)]}{[Prevalence\ in\ Contacts / (1 - Prevalence\ in\ Contacts)]}$

Challenges in Tracing Tuberculosis Contacts - The Ugandan Experience

Margaret Nakakeeto
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Introduction A prospective study to assess the risk of tuberculosis infection in household contacts versus individuals in the community and the infectiousness of patients with sputum smear positive pulmonary TB has been on-going since October 1995.

Objective We examined the major challenges and obstacles to TB contact tracing in a cohort of Ugandan households. Solutions to some of these challenges are subsequently discussed.

Methods A cohort of adult index cases with initial episodes of smear-positive pulmonary TB were identified at the National TB Treatment Centre at Old Mulago Hospital. Their household contacts traced and evaluated for tuberculosis infection and active TB. Possible problems to be encountered were documented prior to beginning the study by creating various scenarios and role playing them. We have continued to document real situations as reported by the home visitors through regular discussions with the team.

Results A number of the challenges and obstacles were revealed. First and foremost, evaluation of households and contacts are carried out in the homes. The most challenging evaluations are vital signs, PPD skin testing,

blood drawing, radiographic and gastric aspirate examinations. Work is carried out in very difficult environment with limited space and privacy. The majority of the households (74%) are located in slums and semi-urban areas. They are small and overcrowded, with an average size of 2.5 rooms and with 5.1 members in a household. More than one-third of the families include more than eight people. Such an environment increases the stigma of tuberculosis within the community. We had attempted to overcome this by transporting families to the clinic for radiographic examinations, and also by the use of vehicles with Ministry of Education (UE) registration numbers. The association of TB with HIV and death sometimes made obtaining consent for HIV testing difficult. Initially only 26 percent of the TB contacts consented to HIV testing. During health education the team strategically began to plan increased emphasis on the need to prevent HIV-infected contacts from getting TB. The proportion of individuals consenting to HIV testing has gradually increased to 46% and it is 89%, while in the control households it is only 15 percent.

Compliance has been very tricky. Contacts, less than 5 years of age, are started on INH preventive therapy. Most times mothers do not see the need for such treatment. Compliance is also affected by low family incomes where 81% earn less than \$160 (USD) a month and therefore there is little money available for regular clinic attendance. Other factors that influence compliance include the relationship of the index case to the head of household. Forty-three percent had little or no relationship at all and therefore tend to leave the homes after diagnosis. Intensified health education and counseling have been used to overcome this challenge.

Taxi/truck drivers (2.4%) and students (35.6%), especially those in boarding schools, were difficult to trace. Mention of terminating these families improved compliance. Mothers coming together at the clinic encourages peer education and also promotes witnessing of other children who may be sick. This improved compliance tremendously.

Tracing bias in household contacts also was a challenge to us. At month 3 or 12 follow-up,

59.3% of the study group were in contact with another recent TB case. Twenty-three percent of these other TB cases were not on treatment while 27.3% had no evidence as to whether they were on treatment. Other challenges included broken homes, denials, community disruptions, etc.

Conclusion Team work has been the cornerstone to the success of this study. Good health education, counseling, and clear communication given in the right way and at the right time does influence outcome in such a study.

Tuberculosis in Ugandan Children

*Philippa Musoke-Mudido
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The Impact of HIV on Transmission of Tuberculosis

*David Guwatudde
Makerere University*

Objective To examine the effect of HIV co-infection on the infectiousness of TB.

Design Cross-sectional prevalence study of household contacts of patients with smear positive TB.

Setting & Participants HIV-infected and HIV-non-infected index cases with sputum smear positive tuberculosis, living within a radius of 20 km from Kampala city center, were identified at a major TB clinic at Old Mulago Hospital in Kampala, Uganda. All full-time household contacts of the index case were eligible for evaluation.

Measurements All contacts were evaluated by medical history & physical examination, Mantoux PPD skin tests (which were read within 48-72 hours), a chest x-ray, BCG vaccination status, HIV test (among consenting subjects) and presence of any TB symptoms. A contact with signs and/or symptoms of TB underwent a complete work-up for active TB. If active TB was diagnosed, the subject was treated with standard short-course TB chemotherapy (2EHRZ/4HR). In addition, demographic characteristics and household

environment were measured. Measurements were made using standardized interview and review of clinical, radiographic and microbiologic findings by senior physicians.

Main Outcome Measures Tuberculin skin test reactivity, and active tuberculosis. A PPD skin-test reading ≥ 5 mm was considered positive.

Results 147 index TB cases and their 592 household contacts were included in this analysis. Seventy-six (52%) of the TB index cases, were HIV-positive. No significant differences were noted in PPD-positivity between contacts of HIV-infected index cases and those of the HIV-non-infected index cases [adjusted Odds Ratio (OR) = 1.1, 95% CI 0.70-1.73]. Significantly higher levels of PPD-positivity were noted in contacts of index cases with cavitory disease than in contacts of index cases without cavitory disease [OR = 2.2, 95% CI 1.38-3.52]. HIV-infected contacts were less likely to be PPD-positive than HIV-non-infected contacts [OR = 0.3, 95% CI 0.11 - 0.85]. PPD-positivity was significantly higher in contacts of index cases with higher AFB smear grade, being highest in contacts of index cases with 3+ sputum smear [OR = 3.5, 95% CI 1.55 - 7.70].

There was no significant difference in the prevalence of active TB in contacts of HIV-infected versus HIV-non-infected index TB cases. Younger contacts were at a higher risk of developing active TB than older subjects [OR = 0.9, 95% CI 0.85 - 0.96]. HIV-infected contacts were at increased risk of active TB [OR = 14.9, 95% CI 3.56 - 62.5]. Spouses of index cases also were at significantly higher risk of developing active TB than other contacts in the households, [OR = 8.99, 95% CI 2.07 - 38.9]. Sixty-nine percent of the spouses of HIV-positive TB index cases were also HIV-positive, compared to 9 percent of the spouses of HIV-negative index cases.

Conclusion Transmission of *M. tuberculosis* within households was comparable among HIV-infected and HIV-non-infected TB cases. However, transmission differed depending on whether the index case had cavitory disease, the sputum AFB smear, age & HIV-infection status of the contact. HIV-infected household contacts,

children and spouses of the index cases were at increased risk of having active TB at the time of household evaluation

Molecular Epidemiology of Tuberculosis in Uganda

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In Uganda where both TB and HIV are highly prevalent, transmission of MTB is thought to be frequent. In order to gain an insight into the transmission of TB in Kampala, the extent of strain diversity and degree of clustering of MTB isolates from Kampala, Uganda, was examined

A total of 73 isolates from patients with initial pulmonary TB, selected on the basis of geographical location, was DNA finger-printed using IS6110. The mean age of patients was 29 years, range 18-46 and 64 (88%) were HIV seropositive. Of 73 unique strains, 70 different DNA hybridizing patterns were observed. Of 70 patterns, 67 patterns (91.7%) were observed only once. There were three genuine clusters of two isolates that had identical patterns. This DNA fingerprint data suggest that multiple strains of tuberculosis are present in Kampala and may have been transmitted over a long period of time

Cytokine Regulation of the Host Response to *M tuberculosis* infection

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Infection with *M tuberculosis* induces a multifaceted host response which includes macrophage and lymphocyte activation and production of inflammatory and immune stimulatory and suppressive cytokines. Central among these cytokines are inter-leukin-2 (IL-2) and interferon- γ (IFN- γ), two of the mediators of the TH1 protective immune response. TNF- α and other pro-inflammatory macrophage cytokines are the mediators of the host inflammatory response. These molecules induce leukocyte migration and granuloma formation at the site of infection as well as causing symptoms of disease (fevers, night sweats, weight loss) and

tissue damage. Our hypothesis is that the balance between the levels of the protective TH1 cytokines and inflammatory cytokines such as TNF- α determines whether the host will develop active disease and/or severity of disease following *M tuberculosis* infection

To investigate the interaction among these cytokines, we are taking three different approaches. First, using in vitro models of *M tuberculosis* infected human monocytes, we are studying the relationship among the following factors: phagocytosis of mycobacteria, growth and survival of intracellular mycobacteria, and the cytokines and oxidative intermediates produced by the monocytes in response to the infection. Our second approach involves the use of two animal infection models. In the first, mice are infected with aerosolized virulent *M tuberculosis* and the host response both in the lungs and systemically is evaluated. Granuloma size and cellular composition, cellular immune activation, and bacillary load are evaluated over time. This model enables us to manipulate the levels of specific cytokines to determine the relative contribution of particular cytokines to disease outcome. In addition, rabbits are infected intrathecally with virulent *M bovis* to establish a central nervous system infection. The role of cytokines and cytokine modulation in this experimental mycobacterial meningitis model are currently under study. Our third approach is to investigate the cytokine response in patients with tuberculosis and to determine the impact of cytokine modulatory therapy on bacillary load, radiologic characteristics, patterns of immune activation and clinical outcome.

Using this triple pronged approach, we have shown that high levels of TNF- α production induce immunopathogenic effects which are detrimental to the host. Furthermore, increased IL-2 or IFN- γ levels are associated with a reduction in the bacterial load. These cytokines appear to modulate the levels of pro-inflammatory cytokines, thereby improving clinical outcome. The molecular mechanisms underlying these effects are under investigation

Cytokine/Chemokine Cascades in the Immunopathology of Tuberculosis

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The cytokine response in mice infected in the lungs with *Mycobacterium tuberculosis* can be operationally divided into four distinct phases. In the first, pro-inflammatory cytokines produced by infected macrophages, in concert with vasoactive amines and prostaglandins, promote relaxation of the local capillary vessels and the influx of tissue fluid into the interstitium. In phase two, macrophages produce TNF, which in turn induces the production of MCP-1 and other chemokines, initiating the granulomatous response. Production of IL-12 by infected macrophages begins and may be amplified by "innate" IFN produced by NK cells and $\gamma\delta$ T cells. This drives the effector phase, phase three, in which sensitized CD4 T cells enter from the blood and saturate the local tissues with IFN, leading to control and containment of the infection. In phase four, feedback loops come into play, a reduction in key antigen production is further stimulated by the autocrine production of IL-10, TGF- β , and IL-12 homodimer. The production of IL-4 also rises at this time, but does not interfere with any residual protective T cell immunity.

Mice and guinea pigs develop similar levels of immunity to tuberculosis in the lungs, but whereas mice develop a micro-necrotic response over the long term characterized by substantial fibrosis, guinea pigs instead develop caseous necrosis which mineralizes or can even cavitate. In this presentation I propose a new hypothesis that holds that the basis of this susceptibility to overt disease resides in a failure by sensitized T cells in the guinea pig to adequately invade the developing granuloma, staying instead in a mantle outside the epithelioid macrophage fields. In contrast, T cells in the mouse create invasive "wedges" of large numbers of lymphocytes deep into the macrophage field, thus presumably saturating these tissues with IFN. This does not happen to macrophage field centers in the guinea pig, and so they cannot prevent bacterial spread and thus degenerate. I propose that the basis of this phenomenon is the lack of production of, or failure by guinea pig T cells to respond to, local

chemokine signals, or alternatively, the failure of these cells to cross the extracellular matrix of the granuloma, suggesting an integrin deficiency.

Cytokine Profiles in Tuberculosis

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Infection with *M. tuberculosis* (MTB) remains a major health issue worldwide. Despite advances in diagnosis and treatment, the incidence of active TB is increasing worldwide, with the majority of cases occurring in the developing world. Factors that affect TB control relate to the long duration of treatment and the poor compliance with drug therapy. In depth understanding of the human immune response during active TB and following successful therapy of infection with the causative organism MTB, therefore, may be particularly helpful in the design of new therapeutic approaches for anti-tuberculous therapy including immunotherapy.

Existing studies of anti-tuberculous immunity, even though hampered by small sample size and in-homogeneity of patient populations tested (particularly extent of disease, degree of sputum smear positivity, duration of treatment and HIV status) have identified a relative depression of the TH1-type of T-cell response characterized by depressed T-cell blastogenesis and production of IL-2 and IFN- γ , and overproduction and/or enhanced effects of immunosuppressive molecules such as TGF- β and IL-10. Further, recent evidence also suggests that IL-12, a cytokine implicated to be involved in the development of a TH1 type response in naive T-cells, when added to peripheral blood mononuclear cells (PBMC) from patients with active TB *in vitro* augments MTB-antigen-stimulated production of IFN- γ and T-cell proliferation, even though levels of immunoreactive IL-12 do not differ significantly between patients with active pulmonary TB and healthy endemic tuberculin-reactive controls.

To further address these issues, we initially recruited 25 HIV-uninfected and 18 HIV-infected patients with smear-positive, culture confirmed pulmonary TB and 18 HIV-negative healthy

tuberculin-reactive controls. Of the HIV-uninfected patients with TB, four had minimal disease (as assessed by criteria of the National Tuberculosis and Respiratory Disease Foundation), six had moderately advanced disease and 15 had far advanced disease. Of the 18 individuals co-infected with HIV and TB, 6 were classified as moderately advanced disease and 12 as far advanced TB. We assessed MTB-antigen-stimulated production of cytokines (IFN- γ , IL-10, TGF- β and TNF- α) (indicating macrophage activation) in PBMC obtained from individuals in all three groups and also evaluated the effects of co-culture with neutralizing antibodies to TGF- β and IL-10 and recombinant IL-12, alone or in combination with anti-TGF- β and/or anti IL-10. Our results indicate that IFN- γ produced by PBMC from patients with TB stimulated with MTB-antigen *in vitro* was depressed and that both TGF- β and IL-10 were produced in excess in patients with active pulmonary TB when compared to healthy controls. When comparing individuals with or without concomitant HIV-infection, suppression of IFN- γ production was most profound in persons dually infected with HIV and MTB, and levels of the immunomodulatory cytokines TGF- β and IL-10 were increased above those encountered in PBMC culture supernatants from patients with TB only. However, neutralizing antibodies to IL-10 and TGF- β were less effective in reconstituting the IFN- γ response in dually infected persons when compared to individuals with TB only. In contrast, even though levels of immunoreactive IL-12 (p70) did not differ significantly between patients with TB (HIV-infected or not) and healthy tuberculin reactive controls, co-culture with recombinant IL-12 was highly and equally effective in boosting production IFN- γ by PBMC from HIV-infected and -uninfected individuals stimulated with MTB antigen *in vitro*.

Since patterns of cytokines produced by MTB-antigen - stimulated PBMC from HIV-infected and -uninfected persons with TB *in vitro* closely resemble one another, we collected longitudinal follow-up data only in patients without concomitant HIV infection.

We found that levels of immunoreactive IL-10 and TGF- β , induced by stimulation of PBMC with

MTB antigen, returned to baseline by 6 months (completion of short-course chemotherapy), whereas levels of IFN- γ remained depressed for more prolonged periods. In fact, at the time of the 18 months follow-up MTB-antigen-induced production of IFN- γ by PBMC from patients with TB was still lower than that of PBMC from healthy controls.

These data indicate that the immunosuppression of TB is not only immediate, but also long-lasting. Identifying mechanisms that could account for this prolonged T-cell hypo-responsiveness, may elicit new targets for adjuncts to anti-tuberculous therapy.

Immune Activation, and Mortality in HIV-Positive Tuberculosis. Predictors of Outcome and Strategies for Intervention

Robert Wallis

Case Western Reserve University

Co-infection with HIV-1 affects outcome in pulmonary tuberculosis. Immune mechanisms triggered by *M. tuberculosis*, including cellular activation and cytokine induction, may lead to increased cellular susceptibility to HIV infection, and increased HIV expression by cells which are already infected. TNF- α , a cytokine which is produced by macrophages in response to mycobacterial proteins, and which promotes HIV expression in cultured cells, may play a pivotal role in this process, which ultimately may result in accelerated HIV disease progression and shortened survival.

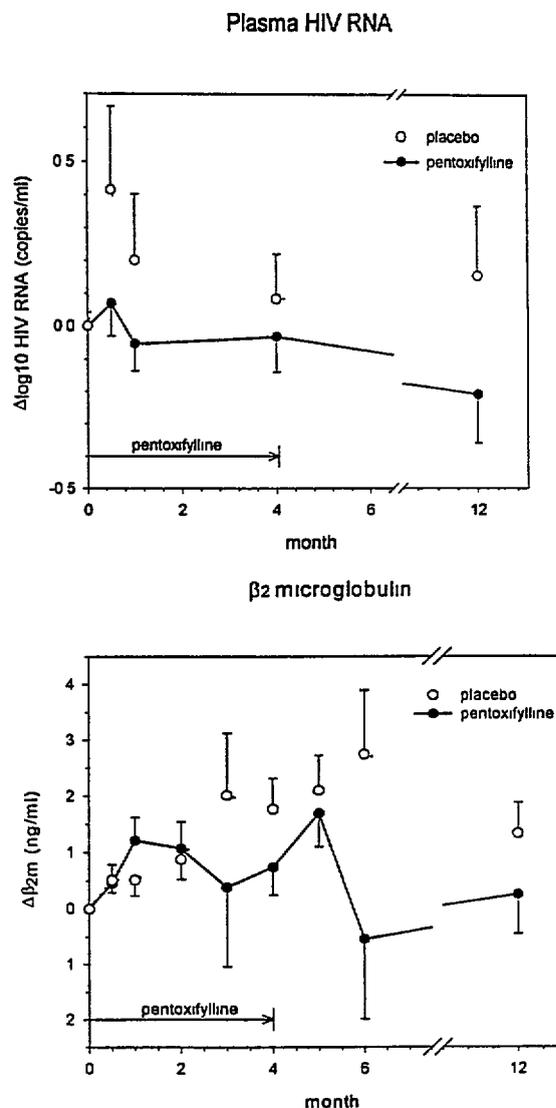
To test this hypothesis, a study was conducted to correlate markers of immune activation with mortality and drug toxicity in HIV \pm TB. Subjects were drawn from a randomized trial of isoniazid - streptomycin - thiacetazone (STH) vs isoniazid-rifampin-pyrazinamide (RHZ). These regimens differ substantially in sterilizing activity, in that after 2 months of treatment, only half of STH-treated patients have converted their sputum cultures to negative, vs >80% for RHZ. This offered the opportunity to examine the differential effects of these regimens on markers of HIV disease progression during TB treatment.

Serum neopterin ≥ 14 ng/ml, TNF- α receptor II ≥ 6.5 ng/ml, and negative tuberculin skin test were independently associated with increased mortality ($P < .01$). In HIV+ patients with TB, the skin test primarily reflects CD4 cell number, and thus is an indicator of current HIV disease stage. Both neopterin and type II TNF receptors primarily reflect macrophage activation, and predict HIV disease progression. The effect of neopterin on survival in HIV+ TB was only evident in skin test positive subjects, i.e., those with early HIV disease, as shown below.

Among STH-treated subjects, dermatologic toxicity and mortality were respectively 13- and 6.3-fold more likely to occur in subjects with elevated neopterin ($P < .05$), although these two adverse events occurred independently. Activation markers increased from baseline after 2 months of therapy with the less-rapidly-bactericidal STH regimen, whereas they declined in those treated with RHZ, suggesting a relationship to continued mycobacterial replication. In subjects with elevated neopterin, survival was significantly shortened in subjects treated with STH, as shown on the following page.

In a second study, the TNF- α inhibitor pentoxifylline, or placebo control, were administered as adjunctive therapy to patients beginning treatment for pulmonary TB with standard therapy (HREZ). In control subjects, plasma HIV RNA and β_2 microglobulin (a marker of immune activation reflecting both T cell and macrophage activation) rose during therapy and remained elevated during the period of observation. In contrast, this increase was blunted in subjects treated with pentoxifylline 1800 mg/day, as shown below. The difference between active and placebo arms persisted beyond the period of drug administration, suggesting that intervention during a period of maximum inflammation might offer prolonged benefit. In a subset of moderately anemic patients, pentoxifylline also resulted in improved blood hemoglobin. Trends were noted toward reduced TNF- α production in vitro and improved performance scores, but these did not reach statistical significance. No effect was noted on body mass, CD4 cell count, or survival.

These studies suggest immune activation in HIV+ TB is associated with shortened survival and increased risk of drug toxicity. HIV+ TB patients, particularly those with elevated serum neopterin, should be treated with a rapidly-bactericidal drug regimen which does not include thiacetazone.



However, even optimal treatment of TB in an HIV+ patient appears to result in sustained increases in HIV expression as a consequence of immune activation. Although the effects of the cytokine inhibitor pentoxifylline on this process are statistically significant, they are small and of uncertain clinical significance. Additional studies of more potent TNF- α inhibitors, such as prednisone or marimastat (a TNF- α convertase inhibitor), are warranted in HIV+ tuberculosis.

Surrogate Markers and Response to Treatment of Tuberculosis

Moses Joloba
Makerere University

Standard methods, such as qualitative cultures on solid media, for assessing response to anti-TB treatment require up to 6-8 months for final results. New methods which would allow more rapid assessment of drugs and treatment regimen would be very valuable. A study to develop new methods and surrogate markers of antibacterial activity in TB patients was performed. This was a prospective multi-center study involving HIV negative adults with initial episodes of recently diagnosed sputum smear positive TB patients in Vitoria, Brazil, New York and Kampala, Uganda. All patients were treated with the standard short-course therapy (2HRZE/4HR). Spot sputum specimens were homogenized by N-Acetyl-L-cysteine digestion and vortexing with glass beads. Aliquots for alpha antigen detection by ELISA, quantitative IS 6110 PCR, rRNA and mRNA were frozen stored. 2.5 mls of digested sputum was decontaminated by the NaOH method and centrifuged. The sediment, after being reconstituted to the original 2.5mls using phosphate buffer, was used to inoculate Bactec 12A bottles, 7H10 plates, slides and to make dilutions for quantitative colony counts.

A total of 23 patients were recruited in Uganda, 21 in Brazil and 20 in New York. The mean age of patients was 27 (range 18 - 43 years (SD = 8 years)). The study demonstrated a 1 log₁₀ CFU fall by day 4. Most of the patients were culture negative by two months and smear negative by three months. Bactec cultures were still positive at 2 months: 17% in New York, 31% in Brazil and 61% in Uganda. In Uganda, only two of the isolates had mono-resistance to INH and RIF the rest were susceptible to all the drugs. There was no difference in CFU curves of mono-resistant strains as compared to the fully susceptible ones. In this study, Early Bacterial Activity (EBA) was demonstrated in three distinct populations of TB patients receiving standard short-course chemotherapy.

Current Status of Vaccination for the Prevention of Tuberculosis

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BCG vaccines are all we now have for immunoprophylaxis against tuberculosis. Produced by several different manufacturers, using slightly different seed strains and different protocols, these vaccines are the most widely used of all vaccines today, being administered to almost 100 million children each year. They are also the most controversial of all today's routine vaccines. Though the WHO/GPV recommends only a single dose in infancy, many countries have unique schedules (for example the vaccines are recommended for tuberculin negative adolescents in the United Kingdom). Only the USA and Holland have never recommended their universal use, and some northern European nations such as Sweden have recently restricted their use to high risk groups alone on the basis of reduced cost/benefit ratios under conditions of low tuberculosis incidence.

The major controversy surrounding BCG vaccines concerns their efficacy against pulmonary tuberculosis. A dozen controlled trials and more than 30 observational studies have provided estimates of efficacy ranging from nil to 80 percent. There is significant heterogeneity in these results, indicating that the differences reflect biological factors and not just sampling errors. A large literature discusses various hypotheses for the observed differences, which have been attributed to strain differences between BCGs, to genetic differences between human populations, to geographic differences in the tubercle bacillus, to ecological factors (e.g. sunlight), to nutrition, and to immunological interaction or masking by heterologous immunity attributable to exposure to various environmental mycobacteria. Though it is possible that each of these factors has some influence, it is now indisputable that geographic differences in exposure to environmental mycobacteria are a major contributor to the observed variation. Among the lines of evidence supporting this conclusion are experimental studies in mice and guinea pigs, and a significant tendency for lower efficacy in lower as compared to higher latitudes, which is thought to reflect the higher prevalence and variety of environmental

mycobacteria in the warmer and wetter regions of the world

Several studies have examined the efficacy of BCG vaccines against leprosy. Not only have these studies all shown some protection, but the evidence to date suggests that protection is consistently greater against leprosy than against tuberculosis. This is an important observation, first because it justifies the continued use of BCG vaccines in tropical countries, even where they may provide little protection against pulmonary tuberculosis, and second because it indicates a paradoxical species specificity in BCG's action - in effect the vaccines appear to be more affective against more distantly related *M leprae* than against the more closely related *M tuberculosis*. However, given that BCG vaccines appear to protect more strongly against non-pulmonary tuberculosis (in particular meningitic and miliary disease) than against pulmonary disease, this difference may reflect organ-specificity rather than mycobacterial species specificity. The immunological implications of these differences have yet to be worked out, but they may well provide important clues which will be useful in the development and evaluation of any new anti-tuberculosis vaccines.

Looking beyond efficacy, the public health impact of BCG vaccination against tuberculosis is extremely difficult to assess, in particular as its introduction typically coincided with other interventions (improved case finding and drug regimens, as well as general socio-economic improvements) each of which should have had some influence on tuberculosis incidence. The recent tremendous impact of HIV upon tuberculosis incidence has further complicated the issue of evaluating the overall influence of BCG.

BCG is generally contraindicated in HIV positives in developed countries, but the contra-indication is restricted to individuals with clinical AIDS in most developing countries (where BCG vaccination is restricted to infants). Given the high prevalence of HIV infection and AIDS in many countries, in particular in Africa, and the fact that many millions of HIV-affected individuals have a history of BCG vaccination, the rarity of recognised systemic BCGosis provides evidence that the BCG bacillus does not persist in vaccinees. This too is an

important observation, because of its implications for memory of anti-mycobacterial immunity.

Rather than despair over BCG's paradoxical, apparently unpredictable and incessantly controversial behaviour, we should examine closely this accumulated experience, as it provides valuable clues to the nature of mycobacterial immunity and to possible ways to develop an improved vaccine against tuberculosis.

Development and Testing of New Tuberculosis Vaccines

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Novel Strategies for TB Control Finding Gold in Soil and Cow Dung

William Jacobs
Albert Einstein University

Novel strategies are currently needed to control the global spread of tuberculosis. The inability to perform genetic analysis has precluded the acquisition of much basic knowledge of *Mycobacterium tuberculosis*. For example, five years ago none of the enzyme targets of mycobacterial-specific drugs isoniazid (INH), ethionamide (ETH), ethambutol, pyrazinamide or para-salicylic acid were known. Over the last ten years, we have used mycobacteriophages as tools for developing genetic systems for mycobacteria. These systems allowed us to identify genes involved in tuberculosis virulence and allowed for identification of genes encoding targets for INH, ETH, and ethambutol. The knowledge of a target for both INH and ETH, has allowed for an understanding of a mechanism of action of these drugs and also, the development of drugs active against INH-sensitive and INH-resistant *M tuberculosis*. Phage vectors have also been used to generate luciferase reporter phages which have the ability to efficiently allow *M tuberculosis* cells to emit light and provide methodology for rapid detection of drug susceptibilities. Lastly, recombinant mycobacteriophages have been used to develop efficient transposon delivery systems for the identification of genes responsible for virulence. Knowledge of the virulence

process should lead to the development of novel interventions and vaccines

BCG Case-Control Study in HIV-infected Women

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Programme*

A case-control study was conducted to investigate the effect of HIV infection on the protective effect of BCG vaccination for tuberculosis in Uganda. A variety of reports have suggested that HIV infection may interfere with the protective efficacy of BCG vaccination by suppressing the immune system of an individual leading to reactivation of latent TB infection. The study was conducted in Mulago Hospital, the National Referral Hospital situated in Kampala.

From October to December 1990, data on all incident and bacteriologically proven cases of TB identified in Mulago Hospital from January 1989 to December 1990 was collected by review of medical records, personal interviews and physical and laboratory examination. The cases were then frequency matched by sex and age to controls enrolled from the roster of a current study in Mulago Hospital on the "Transmission and course of HIV infection in Infants".

A total of 196 cases, 112 females and 84 males were enrolled and matched to 304 controls, 278 females and 26 males. Analysis is based on the 112 female cases and 255 female controls who met the entry criteria and had complete information.

Univariate results demonstrate a protective effect of BCG vaccination of about 59% (95% CI 33-75%). Stratification by HIV status showed a markedly reduced protective effect of BCG in the HIV positive individuals, 42% (95% CI 0-67%) as compared to 83% (95% CI 55-94%) among those who are HIV negative. In addition, results stratified by, age by five years, show a protective effect of BCG of 61% (95% CI 36-76%), education level 56% (95% CI 28-73%), parity 61% (95% CI 37-76%) and by marital status, 59% (95% CI 2-83%) for single women, 42% (95% CI 0-72%) for married women and nil for

widowed or divorced women. Stratification by job status demonstrated a protective effect of BCG vaccination of 67% (95% CI 0-97%) for the employed, 55% (95% CI 0-90%) for the self-employed and 65% (95% CI 38-80%) for the unemployed.

These important findings offer major implications on the role of BCG vaccination as a tool for control of tuberculosis during this HIV epidemic and what impact the latter will have on the tuberculosis situation in this community. The role of BCG vaccination needs to be critically reevaluated especially in communities like Uganda, where both tuberculosis and HIV infection are independently prevalent.

The study is to be extended to meet the anticipated sample size of about 200 cases and 3 to 4 times as many controls and to include generalization to both sexes for more accurate and representative estimates of the protective effect of BCG vaccination and reassess the effect of HIV infection.

***Mycobacterium vaccae* - The South African Experience**

*Bernard Fourie
Medical Research Council - South Africa*

***Mycobacterium vaccae* - The Ugandan Experience**

*Moses Kamya
Makerere University*

M. vaccae is a non-pathogenic mycobacteria which was originally isolated from Uganda soil. It has been studied for its usefulness as adjunctive immunotherapy in the treatment of TB. It has been hypothesized that inoculation with heat killed *M. vaccae* may down regulate tissue-destroying hyper-sensitivity reactions (Th2) associated with cavity formation and progression of disease and activate host protective immune responses (Th1) leading to macrophage activation and granuloma formation. This could potentially hasten the clearance of persisters and lead to shortening of TB treatment regimens.

Objectives 1 To evaluate the short and long-term safety of heat-killed *M. vaccae* immunotherapeutic agent 2 To evaluate the microbiological and immunological activity of *M. vaccae* immunotherapeutic agent as an adjunct to short course therapy in HIV seronegative Ugandan adults with moderate to far advanced fully drug-susceptible pulmonary tuberculosis

Design and Subjects Phase I/II randomized, double blind, placebo-controlled clinical trial 120 HIV-1 seronegative Ugandan adults with initial episodes of sputum smear-positive and culture-confirmed pulmonary tuberculosis were randomized to receive either standard treatment and *M. vaccae* or standard treatment and placebo The first 20 subjects were monitored for short term safety for 8 weeks after vaccination in a phase I study After NIH and FDA review and approval they were rolled over into the phase II stage of the trial and an additional 100 subjects were enrolled Adverse events were graded using standardized toxicity tables and the WHO Cause-Effect Scale was used for attribution of the relationship between adverse events and test article The total duration of study follow-up is 12 months after the onset of TB treatment

Intervention Single intracutaneous injection of 0.1 ml of heat killed *M. vaccae* or placebo (sterile borate buffered saline), one week after onset of short course chemotherapy (2EHRZ/4HR)

Main Outcome Measures 1 Rate of adverse events comparing treatment arms, 2 Rate of sputum culture conversion, 3 Rate of increase in vitro PPD-stimulated interferon-gamma production by peripheral blood mononuclear cells and 4 Rate of decrease in serum IgG and IgM antibodies to MTB culture filtrate

Results 409 subjects were screened for participation in the study One hundred and twenty patients were enrolled The majority of subjects were excluded for HIV-1 seropositivity Eleven subjects were excluded for primary INH resistance All patients have completed at least 6 months and 31 have completed the one year total follow-up period The mean age of the participants is 28 years Mean body weight is 52 kg Eighty-six (72%) are male Ninety (75%) had

far advanced and 30(25%) had moderately advanced disease on chest X-ray Ninety (76%) had cavitory disease

Adverse events have been mainly mild local reactions Swelling at the local site was the commonest local reaction (114/120) and was mostly observed on the day of vaccination Other local reactions included pain/soreness, warmth at site, pruritus, nodule and scar formation Arthralgias of mild and moderate grades were the commonest systemic reactions Most of the arthralgias occurred between the 6th and the 9th week of follow-up and resolved after completion of the intensive pyrazinamide containing phase of TB treatment Four severe adverse reactions (2 deaths, one severe dyspnea and one hospitalization) have occurred The two subjects who died had extensive fibro-cavitory TB and most likely died of underlying TB The subject with severe dyspnea had superimposed pyogenic pneumonia and recovered with antibiotic therapy One subject was hospitalized briefly due to homelessness Culture negativity rates were as follows 33% by one month, 62% by 2 months, 84% by 3 months and 100% by 6 months were culture negative Two subjects were treatment failures and 2 subjects have relapsed after TB treatment All subjects remain in blinded follow-up

Conclusion We conclude that the *M. vaccae* immunotherapeutic agent is well-tolerated and does not appear to interfere with expected treatment response Analysis of the immunologic activity is in progress The study is still blinded and follow-up is continuing

Can New Rapid TB Diagnostics be Applied in Africa

Kathleen Eisenach
University of Arkansas

Early diagnosis of TB and rapid recognition of resistance to the major anti-tuberculosis drugs are of utmost importance in reducing the epidemic spread of the disease In developed countries, the definitive diagnosis still relies on culture of *Mycobacterium tuberculosis* from patient specimens Smears from respiratory specimens are crucially important for a presumptive diagnosis of

TB, as well as for the purpose of monitoring the patient's response to chemotherapy. During the last ten years considerable advances have been made in the TB laboratory with the introduction of fluorochrome staining, the radiometric BACTEC system, DNA probes, and amplification assays.

The simplicity and speed of the acid-fast smear have made this procedure a popular method for detecting active pulmonary TB. Although the acid-fast smear does not distinguish *M. tuberculosis* from other mycobacteria and the overall sensitivity is rather low (50% to 80%), it is considered to be a valuable method for identifying contagious patients. Smear sensitivity is highest in patients with cavitary lesions and poorest in the immunocompromised host or those with extra-pulmonary disease. The sensitivity can be significantly improved with use of fluorescent stains, hence this method has become routine in the clinical mycobacteriology laboratory.

The radiometric BACTEC system has become the "gold standard" culture method for mycobacteria as the recovery rate and time-to-detection are vastly improved over traditional solid media. The average time to detect a positive culture ranges from 8 to 15 days, with heavy smear-positive sputum specimens being positive as early as 1 to 3 days. The Gen-Probe AccuProbe culture confirmation kit has become the leading method for identifying *M. tuberculosis* from primary cultures, either solid or liquid. When used in conjunction with the BACTEC, identification can be shortened by one to two weeks.

For drug susceptibility testing, the BACTEC and agar proportion methods are most commonly used. With either method results can be obtained sooner when the test is performed directly on the processed clinical sample. Success of the direct test depends on having a sufficient number of AFB in the inoculum, thus only smear-positive specimens can be used. Again the advantage of the BACTEC system is rapid results which are available within 4 to 6 days after inoculation of drug-containing media.

Alternative culture systems (Becton Dickinson BACTEC 9000MB, Organon Teknika MB/BacT) are now available which offer non-radiometric, automated methods with sensitivity and time-to-

detection essentially equivalent to the BACTEC 460. These second-generation broth systems also have the potential to perform drug susceptibilities.

The most dramatic improvement in the rapidity and accuracy of TB diagnostics has been due to the application of molecular biological techniques. The most significant advances have been made by amplification techniques targeting mycobacterial DNA or RNA. Currently, most experience is available from the direct detection of *M. tuberculosis* complex in respiratory specimens, in particular with the PCR amplification of the insertion element IS6110. More recently, two commercial assays (Gen-Probe AMTDT, Roche AMPLICOR MTB) are available for use in conjunction with smears to aid in the initial diagnosis of TB. The Gen-Probe AMTDT features transcription-mediated amplification of the 16S rRNA and the AMPLICOR MTB amplifies by PCR a region of the 16S rRNA gene. All of these assays perform best (high sensitivity and specificity) with smear-positive specimens from untreated patients. There is considerably less experience with direct detection in non-respiratory specimens. Some of the research-based PCR assays demonstrate high sensitivity with samples containing low numbers of *M. tuberculosis*. When the Gen-Probe AMTDT was adapted for the detection of *M. tuberculosis* in non-respiratory samples, the sensitivity and specificity were 85.7% and 100%, respectively. The few studies performed to date indicate that the AMTDT could be applied to the diagnosis of extra-pulmonary TB.

Can these diagnostic methods be applied in developing countries, such as Africa? It seems unlikely that most of these "high tech" methods would be applicable in the hospitals, clinics and field settings in low-middle income countries. However, one can envision that they could be available in centralized reference laboratories and the testing utilized for special situations. Currently, many developing countries have TB BACTEC systems in university hospitals or federal reference centers. These labs routinely culture specimens from TB patients to obtain isolates for subsequent drug susceptibility testing. In many research laboratories there are PCR thermal cyclers being used for molecular diagnostics for TB and other infectious disease agents.

While the trend is toward more sophisticated methods of detection and identification, there are many instances where conventional methods are sufficient and even preferable. Often simplified or alternative methods can be adapted to particular clinic and field settings. In the case of microscopy, use of a fluorescence microscope to increase the sensitivity of the smear may not be feasible. However, other means for concentrating the AFB in the clinical specimen should be considered, i.e., cytocentrifugation or processing with a novel detergent (CB-18, C₁₈-carboxypropylbetaine) which alters cell wall properties and decreases the buoyancy of the organisms. For settings not equipped with a clinical centrifuge or cytocentrifuge, the AFB can be concentrated by overnight sedimentation. This simple procedure involves liquefying the sputum with sodium hypochlorite, which reduces the amount of debris (yielding a clear field for microscopy) and renders the sample noninfectious, leaving it on the bench overnight, and preparing a smear of the sediment. Although the sensitivity is not as great as with centrifugation, it is considerably higher than direct microscopy.

Considering the need to have rapid culture and drug susceptibility results, the new simple liquid culture system, Mycobacteria Growth Indicator Tube (MGIT), from BD is an alternative. With the MGIT system growth is detected visually as positive cultures exhibit a bright orange fluorescence when exposed to a long-wave UV lamp. Advantages of the MGIT are the broth does not contain a radioisotope, no instrumentation is required, and multiple tubes can be inspected simultaneously. The MGIT system is comparable to the BACTEC 460 in terms of the sensitivity and requires a slightly longer time to detect a positive culture. A presumptive identification of *M. tuberculosis* can often be made when the positive culture demonstrates cord formation upon staining. The presence of cord formation in the BACTEC medium as a criterion for rapid identification of *M. tuberculosis* has proven to be reliable.

An innovative approach to identifying *M. tuberculosis* and determining drug susceptibility patterns is the luciferase reporter phage (LRP) assay. The LRP assay when coupled to the BACTEC-NAP (*p*-nitro-*a*-acetylamino-*b*-hydroxypropiophenone) method can readily

differentiate *M. tuberculosis* from other mycobacteria and yield drug susceptibility results in a slightly shorter time than with the routine BACTEC method. The more relevant application of this sophisticated technology will be direct use on clinical samples. Efforts are underway to develop sample processing methods which are suitable for the LRP assay and reporter phages with higher specificity and sensitivity. The prospect of having, in the near future, a reporter phage system that can monitor mycobacterial viability and drug susceptibility directly on clinical samples is very exciting.

Nucleic acid amplification methods, particularly PCR, have made rapid detection of *M. tuberculosis* feasible, but routine drug susceptibility testing still depends on culture methods. Recent major advances have been made in determining the molecular basis of *M. tuberculosis* resistance to isoniazid, streptomycin, quinolones and rifampin, offering the prospect of a genotypic approach for detection of drug resistance. The mechanism of resistance to rifampin has been attributed to point mutations and small insertions or deletions in a limited region of the gene encoding for the β -subunit of the RNA polymerase (*rpoB*). Based on this data, a practical molecular method has been commercially developed (Line Probe Assay, Innogenetics) to rapidly detect rifampin-resistant *M. tuberculosis*. This assay combines nested PCR with DNA hybridization under stringent conditions to detect mutations in the *rpoB* gene. Probes that match the wild type sequence and the most common mutations are bound to a membrane strip. Also, a species-specific sequence that will identify *M. tuberculosis* is immobilized on the strip. When testing clinical samples, results with the PCR-Line Probe Assay are available in less than 48 hr. More experience with this method is needed to evaluate its reliability and usefulness.

In the developed countries the role of the nucleic acid amplification assays is still being defined. Undoubtedly the current commercial assays will not be broadly applied in the mycobacteriology laboratories. The tests might be reserved for high-risk cases, untreated patients with suspicious chest x-rays or relevant clinical histories, and smear-positive specimens. Certainly direct amplification tests, especially when coupled with smear results, will give clinically meaningful preliminary data to

heighten the index of suspicion for disease and to institute appropriate therapy

How the amplification tests will transfer to the developing countries is still an unknown. Also, how the assays will perform in settings where TB is endemic is another important question. Laboratories capable of culturing specimens and performing drug susceptibilities will be able to perform the amplification tests. However, in their present format and current cost, the applications will be limited. One should remain optimistic that a "low tech" test which rapidly identifies *M tuberculosis* and detects resistance to common anti-tuberculosis drugs will eventually be available and can be broadly applied in both developed and developing countries

Advances in the Treatment and Prevention of Tuberculosis

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Short-course therapy with 6-month regimens has been proven effective for the treatment of HIV-infected tuberculosis patients in Africa, although questions about duration of protection from relapse remain unanswered. Indirect evidence suggests that the 8-month, short-course regimen without rifampicin in the continuation phase is also effective, but results from prospective studies are not yet available. The use of long-acting rifamycin derivatives, such as rifapentine, hold the promise for provision of once-weekly treatment. However, an initial study of rifapentine conducted in Hong Kong yielded disappointing results, possibly because drug with suboptimal bioavailability was used. In a current CDC-sponsored trial of once-weekly rifapentine/isoniazid given in the continuation phase, patients with HIV-infection were found to have an increased risk of relapse with acquired rifampicin mono-resistant disease. Before studies of rifapentine are undertaken in Africa, an optimal dose of the drug and regimens which are safe for patients with HIV-infection must be developed. Experimental studies now underway may assist in the design of such studies.

Isoniazid preventive therapy (IPT), despite being proven effective and recommended by WHO and

the IUATLD for HIV-positive, PPD-tuberculin test-positive persons, has not been applied on a programmatic basis in low-income countries. Although this intervention may be cost-effective when given to persons known to be HIV-infected, in most countries in sub-Saharan Africa the cost of providing voluntary counseling and HIV testing to identify IPT candidates in a tuberculosis prevention program is prohibitive. Therefore, IPT may find its most important application as a 'low-cost' standard of care for persons being treated in AIDS care programs. Concerns about misuse of IPT leading to an epidemic of isoniazid-resistant tuberculosis are probably exaggerated, as very simple screening algorithms should identify most persons who require treatment for active tuberculosis. 'Short-course' preventive therapy regimens which include rifampicin have been evaluated in a number of settings during the past decade, and the initial results of these studies have been promising. Of especial interest for 'limited duration' preventive therapy are intermittent regimens with long-acting rifamycins, such as rifapentine or rifabutin. However, these regimens are probably not feasible in areas where rates of tuberculosis transmission remains high and where, therefore, 'life-long' preventive therapy may be desirable.

Rifampicin and Thiacetazone Treatment Regimens in HIV-infected Tuberculosis Patients

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Introduction WHO estimates that more than 5.6 million persons worldwide are dually infected with HIV and *M tuberculosis*. HIV confers the greatest known risk for the development of active TB, by reactivating dormant infection, but progressive primary infection. Prior to HIV pandemic, thiacetazone was widely used in TB treatment in developing countries due to its low cost and availability in a combination formulation with isoniazid (INH).

Rationale The Uganda NTLP recommended standard anti-TB treatment regimen consisted of daily INH and thiacetazone for 12 months supplemented with streptomycin for the initial two months. Between 50-67 percent of adult tuberculosis patients treated in Kampala are co-

infected with HIV. About 20% of HIV infected TB patients on therapy with STH developed mucocutaneous hypersensitivity reactions to thiacetazone in earlier studies. No controlled clinical trial had been performed to compare the safety and efficacy of STH with any other alternative short-course regimen to treat TB in HIV-seropositive individuals.

Specific Aims To evaluate the safety and efficacy of 2HRZ/7HR compared with STH/10TH in HIV-infected adults with newly diagnosed initial episodes of sputum smear-positive pulmonary TB.

Methods A randomized non-blinded clinical trial at the TB Treatment Centre, Mulago NTLF. Subjects were HIV-infected adults with suspected pulmonary tuberculosis recruited from the TB Treatment Centre. Regimens included 2HRZ/7HR and 2STH/10TH.

Outcome 1) Adverse drug reaction, 2) Response to treatment, and 3) Survival.

Results Between May 1990 and September 1991, 410 patients with suspected TB were screened. 330 out of 410 patients were sputum AFB smear positive. 218 out of 330 were HIV-1 positive and 191 patients were randomized to receive either 2HRZ/7HR or 2STH/10TH. 13 adverse reactions occurred, 1 in RHZ arm (1.6 per 10 PYO) vs 12 in STH arm (18.2 per 100 PYO). Ten cutaneous reactions occurred in STH compared with 1 in RHZ. Sputum AFB were significantly reduced in RHZ (90%) vs STH (76%) ($P < 0.05$) after two months of TB treatment. Sputum culture negativity rates at 2 months were 74% for RHZ vs 37% for STH ($p < 0.001$). Overall survival was similar in both groups at 12 months. The relative risk of death in the STH arm was compared with the RHZ arm was 1.57, (95% CI, 1.00, 2.48).

Conclusions The rate of adverse drug reactions in the STH arm was more than 12 times higher than in the RHZ arm. Sputum conversion was more rapid in patients treated with RHZ. RHZ is recommended for treatment of tuberculosis in HIV infected persons.

Role of Ethambutol in the Consolidation Phase of Tuberculosis Treatment Regimens

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Introduction The purpose of this study is to evaluate the safety and efficacy of anti-TB chemotherapy regimen utilizing an ethambutol-containing continuation phase for the treatment of newly diagnosed pulmonary tuberculosis in HIV-infected adults. The aim was to find a suitable alternative to thiacetazone in the continuation phase given the high cost of regimens utilizing rifampicin throughout the continuation phase and concerns about the high frequency of cutaneous hypersensitivity drug reactions in HIV-infected patients treated with thiacetazone-containing regimens.

The following are preliminary results based on an interim analysis including follow-up through July 1, 1997.

Method Three hundred and eighty-five patients from the National Tuberculosis Treatment Centre, Mulago Hospital in Kampala were screened for the study between April 1995 and March 1996. Eligible subjects included 18 - 50 year old HIV-infected adults with newly diagnosed, initial episodes of sputum AFB smear-positive, culture-confirmed, fully drug susceptible pulmonary tuberculosis. All study subjects were treated with the study regimen consisting of 2 months of daily isoniazid (INH), rifampicin, ethambutol and pyrazinamide followed by 6 months of thrice weekly INH and ethambutol (2EHRZ/6H₃E₃) given as modified directly observed treatment. At least two doses of study drug treatment were administered to each patient each week under the direct supervision of a home visitor throughout the 8 month course of treatment.

Results One hundred and thirty-six patients met the initial eligibility criteria of whom 123 were fully eligible after review of initial drug susceptibility testing results. Nine patients were excluded due to initial resistance to INH and/or rifampicin. Twenty-eight subjects were terminated before end of therapy for various reasons [defaulted ($n = 1$), died on treatment ($n = 13$), moved outside the study follow-up radius ($n = 4$), withdrew ($n = 4$), lost ($n = 6$)]

Ninety-five patients completed therapy and 87 were declared cured based on predefined clinical, radiographic and microbiological criteria. The treatment failure rate was 7/95 (7.4%). As of July 1 1997, fifteen subjects had positive cultures after cure of whom 12 have so far been confirmed as relapsed/ recurrent TB during follow-up totaling 88.2 person years yielding a relapse rate of 13.6 cases per 100 PYO.

Secondary Outcomes The 2-month sputum culture negativity rate was 95%. 19 patients died during the first year of follow-up, 2 after relapse, for an all cause mortality rate of 20%. Three patients developed significant adverse reactions during treatment. Only 1 patient required discontinuation of treatment for more than one week. No patient had an adverse reaction to ethambutol.

Seventy-six per cent of all urine INH metabolite tests performed during the course of the study were positive.

Conclusions Treatment with 2EHRZ/6H₃E₃ was well tolerated with few significant side effects. The relapse rate of 13.6/100 PYO is comparable to reported relapse rates with standard thiacetazone-containing regimens in HIV-infected adults, but is higher than more expensive regimens utilizing rifampicin throughout the continuation phase. These data have important implications for TB control programs in developing countries with high TB and HIV infection rates.

Mycobacterial Drug Resistance in Uganda

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Objectives 1) To determine the level of initial anti-tuberculosis drug resistance in Uganda, and 2) To establish a routine system of surveillance for resistance.

Methods Through 1995 sputum specimen samples from consecutively registered patients with pulmonary TB who denied prior TB treatment were obtained from five National TB

and Leprosy Control Programme zones. Outside of Kampala, the zones had to refrigerate specimens for up to one week before transport to the central TB laboratory in Wandegaya, Kampala. Specimens were processed with 4 percent NaOH by the Petroff method and planted on Lowenstein-Jensen (LJ) medium without potato starch. The media were incubated for up to eight weeks before declaring them negative. Positive cultures were identified as *Mycobacterium tuberculosis* on the basis of niacin production and susceptibility to thiophen-2-carboxylic acid hydrazide in LJ. Susceptibility tests were performed using the proportion method on LJ and the following drug concentrations: Isoniazid (H) 0.2 and 1 mg/L, Streptomycin (S) 12 mg/L, Rifampicin [R] 40 mg/L and Ethambutol (E) 5 mg/L. Resistance were defined as growth on the control drug free medium. Samples were sent to Borstel, Germany, for quality control.

Results 785 samples were cultured during the study period. The total contamination rate was 6 percent. The yield on culture was much higher at sites that were readily accessible to the central laboratory. There were delays in transporting samples to the laboratory in some cases due to low numbers of patients at some zones. Of the 435 positive cultures we identified three rapid growers, the rest were identified as *M. tuberculosis*. Analysis done in Borstel on 295 samples indicated a high prevalence of *M. africanum* among these isolates as shown in table 1 below.

Species	Number	Comments
<i>M. tuberculosis</i>	39	
<i>M. africanum</i>	178	
<i>M. avium</i>	1	From Jinja
<i>M. fortuitum</i>	4	
" <i>africanum</i> & <i>avium</i> "	3	From Jinja
No growth	70	
TOTAL	295	

The pattern of initial drug resistance for 332 isolates of *M. tuberculosis*/*M. africanum* is shown in table 2 below. Mixed infections and atypical mycobacteria are excluded.

Site (n)	H n (%)	S n (%)	R n (%)	E n (%)
Fort Portal (24)	1 (4)	2 (8)	1 (4)	0
Jinja (80)	8 (10)	2 (2.5)	2 (2.5)	0
Kampala (88)	12 (14)	8 (9)	4 (4.5)	0
Mbarara (74)	2 (3)	1 (1.4)	1 (1.4)	0
Soroti (66)	10 (15)	3 (3.5)	1 (1.5)	0
Total (332)	33 (10)	16 (5)	9 (3)	0

H Isoniazid, S Streptomycin, R Rifampicin, E Ethambutol

Acquired drug resistance among 50 isolates from patients previously treated for TB ranged between 40-50% for isoniazid, 10-18% for rifampicin and 10-18% for streptomycin. Multi-drug resistance to isoniazid and rifampicin was present in only one isolate.

A comparison of drug susceptibility testing results was made between *M. tuberculosis* and *M. africanum* isolates in table 3 below.

Resistance to	<i>M. tuberculosis</i> (39 strains) n (%)	<i>M. africanum</i> (178 strains) n (%)	p
Isoniazid	6 (15)	24 (13)	0.75
Streptomycin	6 (15)	9 (5)	0.02
Rifampicin	0	0	-
Ethambutol	0	0	-
Isoniazid + Streptomycin	4 (10)	6 (3)	0.08

When results of drug susceptibility testing from Wandegaya were compared to those of the Borstel Laboratory, there was 97%, 100%, 99% and 100% agreement for the sensitive strains to INH, rifampicin, streptomycin and ethambutol, respectively. When resistant strains were compared, the agreement was 88%, 50%, 38% and 100% respectively for INH, rifampicin, streptomycin and ethambutol, respectively. Intra-laboratory difficulties in streptomycin susceptibility testing have recently been emphasized by the WHO/IUATLD Supranational Laboratory Network report.

Conclusion The results here demonstrate a relatively low prevalence of initial drug resistance in Uganda. Multi-drug resistance was rare. With

effective TB treatment, especially with DOTS, it should be possible to maintain these low rates. The results will be discussed in relation to other previous findings and the implications for the NTLF will be emphasized.

Relapses in HIV-infected Adults with Tuberculosis

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Introduction The rising numbers of TB cases in HIV-infected individuals in many areas has raised concerns about the adequacy of current approaches to treatment in HIV co-infected persons. Relapse after treatment is an important measure of the effectiveness of TB treatment regimens and their successful application. HIV-infected patients treated with rifampicin-containing short course regimens have generally responded well to anti-TB therapy, however, an earlier study from Kenya reported a 34-fold increased risk for relapse after treatment among HIV-infected patients treated largely with a standard thiacetazone-containing regimen compared to HIV-non-infected individuals. Factors associated with relapse in the pre-HIV era included age, greater radiographic disease, higher sputum bacillary burden, and persistent cavitory disease after treatment. Further information concerning risk factors for relapse among HIV-infected patients with TB would be useful in modifying treatment and follow-up in high risk individuals.

Table 1 Factors predictive of relapse in pulmonary tuberculosis

Pre-HIV era	HIV infected
<ul style="list-style-type: none"> older or younger age initial sputum viable counts (cfu) initial sputum smear grade initial radiographic severity and cavitory disease two-month sputum AFB smear month of sputum culture conversion or 2 or 3 month culture negativity residual cavitory disease at end of treatment compliance host immunologic status (speculative) 	<ul style="list-style-type: none"> age \leq 30 years CD4 count $<$ 100 μL¹ less than 3 lung zones involved in chest x-ray cutaneous reaction to thiacetazone compliance treatment with rifampicin-containing regimens for $<$ 9 months

Objective Identify risk factors for relapse after TB treatment in HIV-infected patients with pulmonary tuberculosis

Methods Nested case-control study within a randomized clinical trial comparing thiacetazone- and rifampicin-containing TB treatment regimens in one hundred and ninety-one 18-50 year old HIV-infected adults with initial episodes of culture-confirmed pulmonary TB. Patients were treated with 2STH/10TH or 2RHZ/7HR. The initial two months of TB treatment were given supervised therapy on an inpatient ward. The continuation phase was self-administered on an outpatient basis. Subjects were seen monthly in clinic during TB treatment and every three months thereafter. Relapse was defined as the development of active TB after successful initial therapy as indicated two or more of the following: (a) new signs and symptoms of active TB, (b) chest X-ray showing a new infiltrate, cavity or effusion not present on earlier films, (c) positive sputum AFB smear, and (d) positive culture for *M. tuberculosis*.

Results Patients who died during treatment, treatment failures and those lost-to-follow-up were excluded from the analysis. One hundred and nineteen patients who successfully completed initial TB treatment were eligible for the case-control study. Ten patients relapsed during a median follow-up of 22.3 months, seven of whom were initially treated with the STH regimen. Four control patients who did not relapse and were followed for a similar length of time after TB treatment were selected for each relapse case by simple random sampling without replacement.

The incidence rate of recurrent TB in patients treated with STH was 10.1 episodes per 100 PYO compared to an incidence rate of 3.1 cases per 100 PYO in patients treated with RHZ [incidence rate ratio 3.2 (95% CI 0.8, 12.3, $p = 0.09$)] Treatment with the STH regimen (OR = 4.2, $p = 0.08$), age ≥ 30 years (OR = 2.9, $p = 0.16$), and irregular compliance (OR = 3.6, $p = 0.1$) were associated with trends towards increased risk for relapse in a univariate analysis. Anergy, presence of initial cavitory disease, increased severity of disease on chest X-ray, bacillary burden, two

month culture negativity, and persistent cavitory disease after TB treatment did not differ between relapses and control patients.

Conclusion HIV infected patients who are older, poorly compliant, and those treated with thiacetazone-containing regimens may be at higher risk for relapse after TB treatment. Relapse rates were low in patients treated with rifampicin-containing regimens.

Preventive Treatment of Tuberculosis in HIV-infected Adults

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Background HIV infection confers the greatest known risk for development of both reactivation and progressive primary tuberculosis. Once active disease develops in a dually TB/HIV infected person, treatment does not appear to significantly reduce mortality even in the presence of good microbiologic response. The role of preventive therapy in non-HIV infected individuals is well established. This strategy has therefore been proposed as a possible method of tuberculosis control in populations commonly afflicted with dual infection.

Objectives The study was designed to determine the protective efficacy of three regimens of TB preventive therapy in PPD-positive and anergic HIV-1 seropositive individuals.

Methods This was a randomised, placebo-controlled clinical trial of three regimens in 2736 HIV-infected adults recruited at medical clinics, counseling and research centers in Kampala, Uganda. After exclusion of active TB PPD+ participants were randomized to placebo (ascorbic acid $n = 465$), or one of three active arms: 1) isoniazid for six months (6H, $n = 536$), 2) isoniazid/ rifampin given for three months (3RH, $n = 554$), and 3) isoniazid/rifampin/pyrazinamide for 3 months (3RHZ, $n = 462$).

Individuals anergic to PPD and candida antigens were randomized to receive placebo ($n = 323$) or isoniazid for 6 months ($n = 393$). The primary end point was incident TB, secondary outcomes included adverse drug reactions and mortality.

Compliance was assessed through attendance at scheduled visits, urinary INH metabolites and self-report at standard interviews

Results Allocation to treatment arms, incident rates of TB per 100 person years and relative risks (RR) compared to placebo are shown by treatment arm after a mean follow-up of 12.3 months

Study Arm	N	TB Cases	Rate	RR	p
PPD Positive					
Placebo	465	21	3.41	1.00	-
Isoniazid	536	7	1.08	0.33	0.01
HR	554	9	1.32	0.40	0.02
HRZ	462	10	1.73	0.51	0.08
Anergic					
Placebo	323	10	3.06	1.00	-
Isoniazid	393	9	2.53	0.83	0.68

The relative risk of TB in the 6H arm was 0.33, for a protective efficacy of 67 percent and was statistically significant ($p=0.01$). A similar effect was found with the 3RH arm versus placebo, the relative risk was 0.40 for a protective efficacy of 60 percent. However, this narrowly missed statistical significance. RHZ had a protective efficacy of 49 percent and this was not statistically significant. The protective efficacy of isoniazid in the anergic group was 27 percent ($p=0.68$). Common adverse drug reactions included rash, arthralgia and paresthesias. These were most common with HRZ but were well tolerated. Hepatitis occurred rarely with INH, and always resolved with removal of the offending drug.

Conclusion Isoniazid or isoniazid/rifampin preventive therapy reduces risk of TB in PPD+/HIV+ infected Ugandans by over 60 percent for at least 12 months. The protection is achieved with minimal adverse effects in the case of INH. The shorter course multi-drug regimens do offer some protection, but at a cost of more side effects, particularly due to pyrazinamide. Isoniazid did not provide any protection in anergic subjects. Mortality was not influenced by preventive therapy regimens used.

While the benefit observed appears to be clinically relevant at individual levels, the

feasibility of adopting this strategy at the level of national tuberculosis control programs remains to be established, particularly in settings where resources for TB control activities are limited.

NOTES