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AUTOANTIBODIES IN PULMONARY TUBERCULOSIS AND LEPROSY IN BLACK SOUTH AFRICANS

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A dissertation submitted to the Faculty of Medicine, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the Degree of Master of Medicine.

Johannesburg, 1988

DECLARATION

This dissertation is my own work. It is being submitted for the degree of Master of Medicine in the University of the Witwatersrand. It has not been submitted before for any degree or examination in any other university. The clinical data was obtained while I was working in the Division of Rheumatology, Department of Medicine, Johannesburg Hospital, and University of the Witwatersrand.



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BERNARDO LEON RAPOPORT

01-12-1988

.....

DATE

DEDICATION

To -

My wife, Lee-Anne, who made many sacrifices, and without whose help, support and belief, this study would not have been possible.

and

My nephew and godson, Daniel, born on 14 May 1988, in Buenos Aires, Argentina, whose brit milah I could not attend, in order to complete this dissertation.

ABSTRACT

Infections can cause autoantibody production. The purpose of this study was to determine the prevalence of autoantibodies in chronic mycobacterial infections in Johannesburg. Sera from 41 leprosy patients and from 49 untreated and 73 treated tuberculosis patients were tested for rheumatoid factor, antibodies against a panel of nuclear antigens, anticardiolipin antibodies and syphilis serology. The antinuclear antibody was positive in 7.3% of the leprosy group, 6.1% of the untreated TB group and 15% of the treated tuberculosis patients (p=0.0125). Antinuclear antibody positivity correlated with duration of treatment (p=0.025). The antinuclear antibody titres were low and there was no specific pattern. No patients had antibodies against native deoxyribonuclear acid, ribonuclear protein, Ro or La antigens. The rheumatoid factor was positive in 2.4% of the leprosy and 2.7% of the treated tuberculous patients but in none of the untreated tuberculous group. The titres ranged from 40 to 160 international units. Positivity was dependent on the technique utilised, being positive in 21% of untreated TB patients and 4% of the treated TB group when using a second detection system.

Anticardiolipin antibody was positive in 12% of the leprosy patients (p=0.04), 2.4% of the untreated tuberculous, and 9.5% of the treated tuberculous group. None of the patients had thrombotic manifestations associated with the anticardiolipin syndrome.

Syphilis serology was very prevalent in all groups, being approximately 25% in the leprosy and tuberculosis groups and 42% in the healthy control group. However, the anticardiolipin antibodies did not correlate with the Rapid Plasma Reagin Test for syphilis.

Due to their low prevalence and frequency in these chronic infections, these autoantibodies should not lead to confusion in distinguishing these conditions from the connective tissue diseases.

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LIST OF ABBREVIATIONS

AA	Adjuvant Arthritis
ACA	Anticardiolipin Antibodies
AIDS	Acquired Immune Deficiency Syndrome
ANA	Antinuclear Antibodies
Anti-RNP	Anti-Ribonuclear Protein
Anti-Sm	Anti-Smith
Anti-SS-A	Anti-Sjögren's Syndrome-A
Anti-SS-B	Anti-Sjögren's Syndrome-B
BCG	Bacillus Calmette-Guérin
BFP	Biological False Positive
ds-DNA	Double-stranded-Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Sandwich Assay
ENA	Extractable Nuclear Antigen
ENL	Erythema Nodosum Leprosum
FANA	Fluorescent Antinuclear Antibodies
FITC	Fluorescent Isothiocyanate
FTA-Abs	Fluorescent Treponemal Antibody/Absorption
HLA	Human Leucocyte Antigen
IgG	Immunoglobulin-G
IgM	Immunoglobulin-M
INH	Isoniazid
LA	Lupus Anticoagulant
MTB	Mycobacterium Tuberculosis
PBS	Phosphate Buffer Saline
PTB	Pulmonary Tuberculosis

RA	Rheumatoid Arthritis
RF	Rheumatoid Factor
RPR	Rapid Plasma Reagin
SLE	Systemic Lupus Erythematosus
TB	Tuberculosis
TPHA	Treponema Pallidum Haemagglutination

CHAPTER 1

INTRODUCTION

1.1 AIMS OF THE STUDY

Infectious diseases are common in black South Africans, and in particular there is a high prevalence of Tuberculosis (TB) and Syphilis. It has previously been reported that infections are associated with induction of autoantibodies which, with the systemic manifestation of these common conditions, may lead to difficulties in differentiating them from the connective tissue diseases. Although recent reports have suggested that mycobacterial infection may play a role in the pathogenesis of autoimmune disorders (van Eden et al. 1985; Editorial, 1986; Holoshitz et al. 1986; Ottenhof et al. 1986), this study does not aim to establish a cause or relationship but deals solely with the prevalence of these autoantibodies in the chronic mycobacterial infections, TB and leprosy.

The prevalence of circulating Rheumatoid Factor (RF) antinuclear (ANA), anticardiolipin (ACA), anti-double-stranded DNA (ds-DNA) and anti-extractable nuclear antigen (ENA) antibodies in sera of black South African patients with pulmonary tuberculosis and leprosy infections was investigated.

A high proportion of patients with autoimmune diseases seen at the Connective Tissue Disease Clinic at Hillbrow Hospital, Johannesburg, were noted to have serological evidence of past or present luetic infection, causing confusion since the

falsely positive Wasserman reaction is a criteria for the diagnosis of SLE. Syphilis serology was thus performed to investigate the prevalence of such infection in the non lupus population and to assess both the prevalence of biological false positive reactors in chronic mycobacterial infections, and the correlation and cross-reactivity between the Rapid Plasma Reagin (RPR) syphilis flocculation test and the ACA enzyme-linked immunosorbent sandwich assay (ELISA). In the treated TB patients both disease and treatment duration were analysed in order to assess their role in the induction of autoantibodies. The antibody titres were also measured in order to see whether it would be possible on this basis to differentiate mycobacterial infections from the different connective tissue diseases.

Controversy existed in previous reports regarding the prevalence of autoantibodies in mycobacterial infections. In any clinic it is important for the assessment and management of patients that the spectrum of "normal" values is established in the local setting. The development of new techniques and the recognition of new autoantibodies make it worthwhile to relook at the association of autoimmunity and infection.

Finally, studies of ACA in diseases other than Systemic Lupus Erythematosus (SLE) are scanty, therefore the prevalence of ACA and its correlation with thrombotic manifestations were assessed in mycobacterial infections.

1.2 ANTINUCLEAR ANTIBODIES

Hargraves et al. (1948) demonstrated the existence of polymorphonuclear cells containing phagocytosed nuclear material of another polymorphonuclear cell. The test was designated the "L E Cell" because it was primarily detected in patients with SLE. Today the test is mainly of historical interest, and is detected in a number of autoimmune conditions as well as in 50 to 75% of patients with SLE.

ANA may be detected by a variety of immunological techniques such as immunoprecipitation, complement fixation and haemagglutination. However, the immunofluorescent method (fluorescent ANA or FANA), first described by Friou et al. (1958), is the most commonly used technique and provides a sensitive and simple screening test for circulating ANAs in the serum.

ANA patterns which have been characterised by immunofluorescence techniques include:

(i) Homogenous (or diffuse)

(ii) Speckled

(iii) Nucleolar

(iv) Peripheral (or rim).

The homogenous pattern (Figure 1) reflects antibodies reacting with insoluble native deoxyribonucleoprotein.

The speckled pattern reflects antibody reactivity to soluble nucleoproteins, including a group of antigens known as the "extractable nuclear antigens" (ENAs).

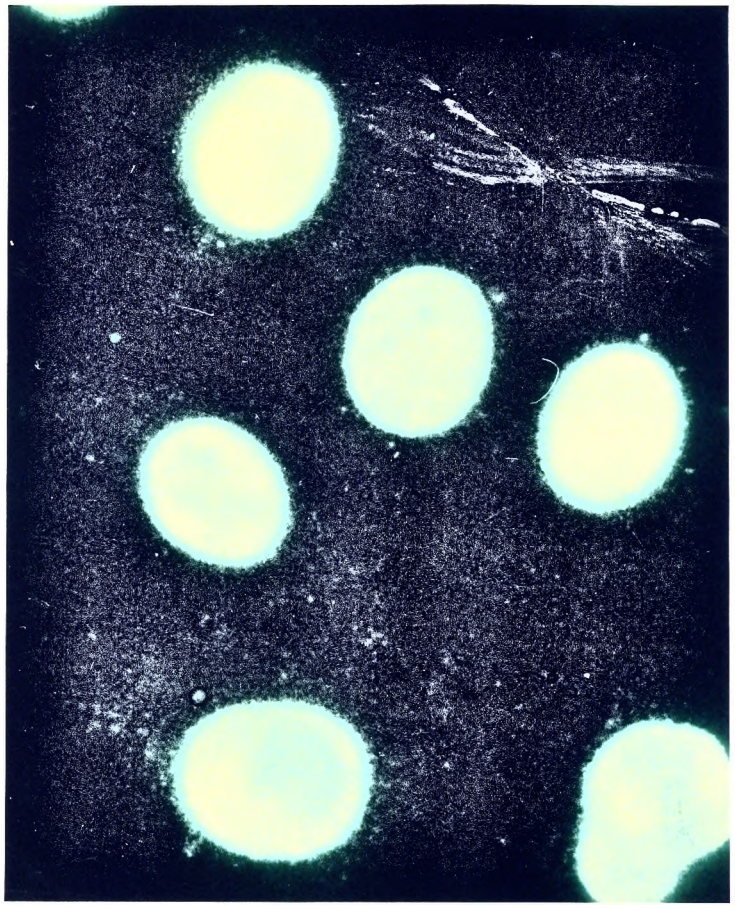


Figure 1 ANA: Homogenous pattern

This is commonly seen in scleroderma, overlap syndromes, mixed connective tissue disease, drug-induced positive ANA, drug-induced SLE and patients with a positive ANA with no clinical evidence of connective tissue abnormality.

A specific speckled pattern is the antibody to centromere (Figure 2). This antibody was first described by Moroi et al. in 1980 in serum of patients with Progressive Systemic Sclerosis. On organ substrates, this antibody gives speckled nucleolar staining. However, on tissue culture cells undergoing methaphase, the speckling can be observed to be associated with centromeres. The nature of the antigen in the substrate is unknown. Presence of antibody to centromere correlates with clinical features of CREST syndrome (calcinosis, Raynaud's phenomenon, esophageal involvement, sclerodactyly and telangiectasia) in 90% of patients (Tan et al. 1980) and has significantly less major organ involvement when compared with other scleroderma patients who exhibit speckled or nucleolar patterns (McCarthy et al. 1983). The nucleolar pattern (Figure 3), commonly seen in patients with scleroderma, may also be present with other ANA patterns (eg. speckled), and is usually due to the involvement of ribonuclear protein.

The peripheral pattern (Figure 4), also known as the 'rim pattern' is associated with antibodies to native and soluble DNA.

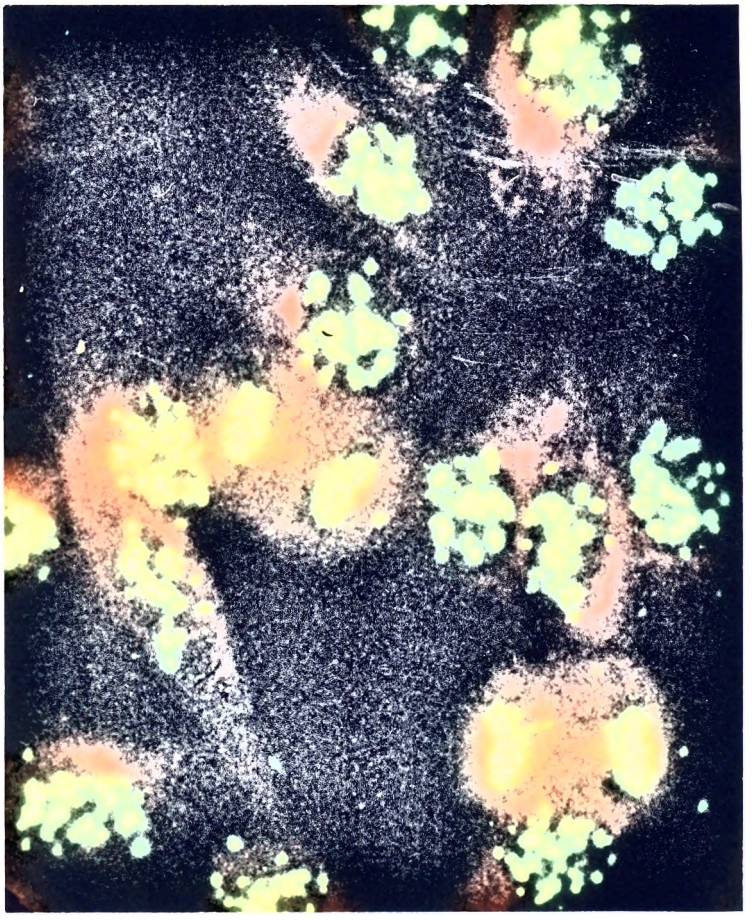


Figure 2 Antibody to Centromere

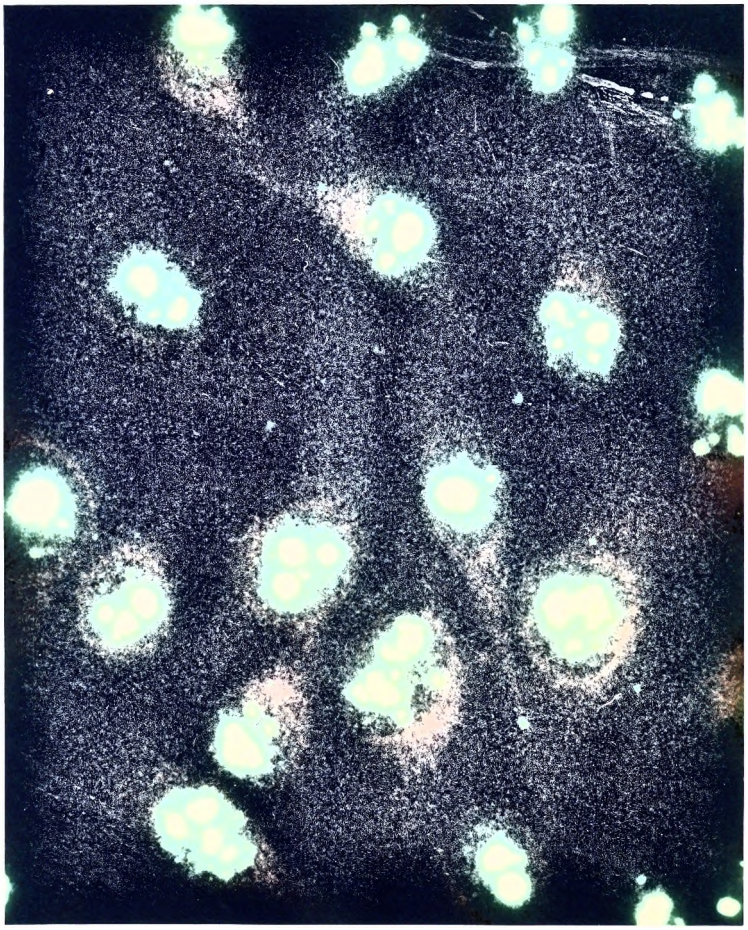


Figure 3 ANA: Nucleolar pattern

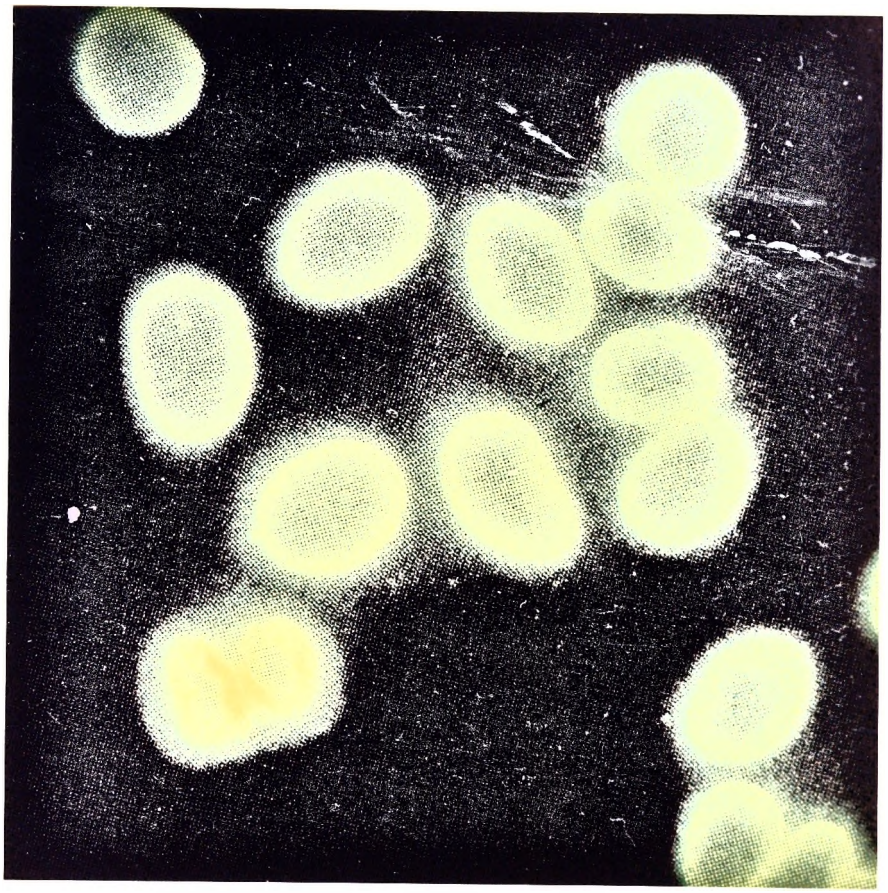


Figure 4 ANA: Peripheral pattern

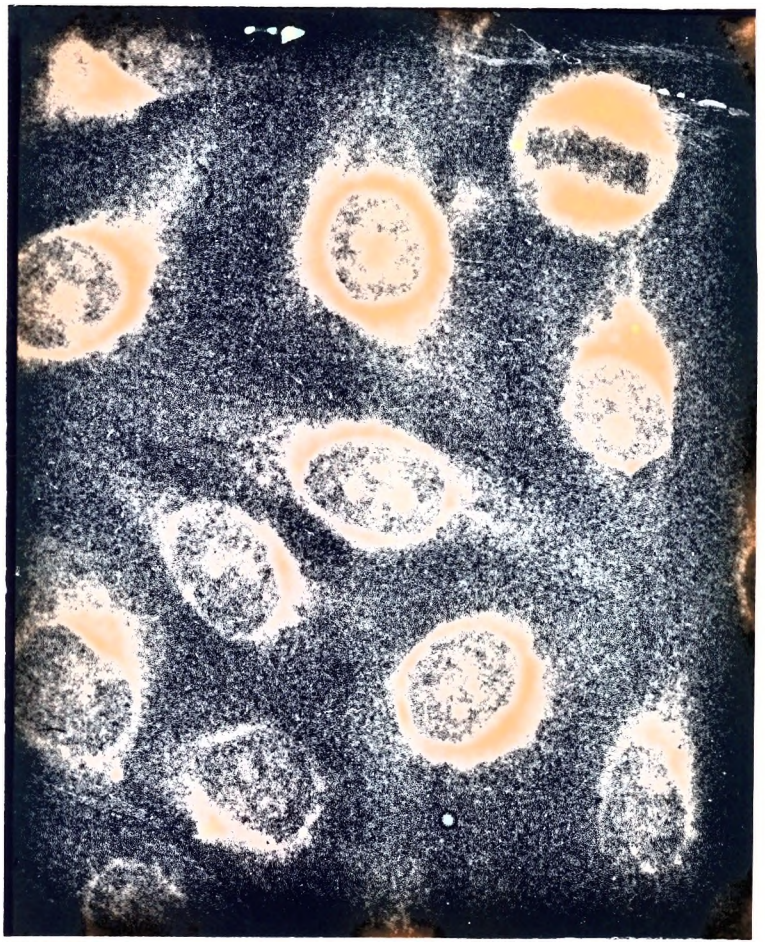


Figure 5 ANA Negative test

1.3 ANTI-HISTONE ANTIBODIES AND DRUG INDUCED LUPUS

The association of drug therapy and systemic Lupus Erythematosus was first reported by Hoffman in 1945. He described a 49 year old patient who developed features of acute disseminated Lupus following treatment with sulfadiazine (Hoffman, 1945).

Eight years later Morrow et al. (1953) reported on 14 of 253 patients treated with 1-hydrazinophthalazine (Apresoline) who developed collagen diseases of varying degrees of severity, all of which regressed on cessation of medication; arthralgias were the most common manifestations. In 16 patients a syndrome indistinguishable from rheumatoid arthritis developed after 4 to 23 months of treatment, and if medication was continued these patients developed SLE.

Alarcón-Segovia (1976) reported that drugs are capable of inducing ANA by two different mechanisms; these are either a pharmacological action or an allergic reaction, and in some cases a combination of both. The first group comprises the larger number of cases, and elicit ANA in a large proportion of individuals who receive them for a specific time period. Furthermore, while on these drugs, more patients develop positive ANA's than drug-induced SLE. In the second group patients develop, after a short period of time, SLE syndromes due to an allergic reaction, which are often acute in onset. However, while on treatment, the patients in this group do not often elicit ANA. Isoniazid (INH) alters the physicochemical

properties of soluble nucleoproteins, resulting in the development of ANA (Alarcón-Segovia, 1976). Drugs included in these groups are listed in Table 1.

Unlike SLE (Tan et al. 1982), there are no definite criteria for the diagnosis of drug induced Lupus (Appendix I). However, the following guidelines should be considered (Hess, 1988): there should be no history of SLE before initiation of treatment with the pharmacological agent; the patient should have a positive ANA test; the patient should have one of the 11 diagnostic criteria for the diagnosis of SLE (see Appendix 1); finally on withdrawal of the drug the patient should have a rapid improvement of symptoms, together with a fall in ANA titres, and no indication of other serological abnormalities.

In this syndrome the central nervous system and renal disease as well as classic malar rash of SLE, alopecia, and discoid lesions are usually absent. Severe haematological abnormalities are uncommon. The serological profile is characterised by a positive LE cell, anti-histone antibodies and ANA tests; normal complement levels and absence of a positive anti-dsDNA antibodies.

The feature of drug induced lupus is the development of anti-histone antibodies. Histones consists of five discrete polypeptides divided into 2 groups. The first group - so called core histone - includes a H2 A-H2 B histone complex as well as H3 and H4 histone. The second group consists of H1 and H5 histones.

Histone H2A and H2B form a complex. Drug induced Lupus is

Table 1 Drugs reported to induce ANA or activate SLE

Group I (by pharmacological action)	Group II (by allergic reaction)
Hydralazine Procainamide Anticonvulsants mephenytoin phenytoin primidone trimethadione ethosuximide carbamazepine pheneturide (phenylethyl acetylurea) Isoniazid Chlorpromazine	Aminosalycilic acid Chlorthalidone * D-penicillamine Griseofulvin Guanoxan Isoquinazepon Levodopa Methyldopa * Methysergide Methylthiouracil Oral contraceptives Oxyphenisatin Penicillin Phenylbutazone Practolol Propylthiouracil Quinidine Reserpine Streptomycin Sulphonamides Tetracycline Tolazamide
* May belong to Group I	

(Alarcón-Segovia, 1976)

characterised by the presence of circulating antibodies to H2A-H2B. However, this is not specific because they are present in 75% of SLE patients (Totoritis et al. 1988).

It was recently reported (Totoritis et al. 1988) that antibodies to H2A-H2B of the IgG subclass are a specific and sensitive marker for drug induced Lupus. In this study the author compared 20 symptomatic and 31 asymptomatic patients treated with procainamide; antibodies to H2A-H2B histone of the IgG subclass was present in all symptomatic patients and in only 2 asymptomatic patients.

The role of the acetylator phenotypes was studied by a number of investigators (Perry et al. 1967; 1970; Price-Evans, 1968; Alarcón-Segovia et al. 1969b; 1971). The compound hydralazine, procainamide and Isoniazid have the chemical commonality of having an aromatic amine or hydralazine group. This suggests that the metabolism of these agents could play a role in the development of drug induced Lupus.

Perry et al. (1967; 1970) showed that patients who are slow acetylators (low levels of acetyltransferase activity) develop ANA sooner than those patients who are rapid acetylators.

Woosley et al. (1978) confirmed these observations in a prospective study where the median time to develop ANA on procainamide treatment was 2.9 months for slow acetylators in comparison to 7.3 months for rapid acetylators. However, Sonnhage et al. (1979) studied 28 patients receiving procainamide prospectively for one year. The dose was graded by

monitoring plasma levels. Thirty percent of patients developed drug induced Lupus. However, there was no statistical difference between slow and rapid acetylators to develop this syndrome.

In the 1970s a total of more than 40 drugs have been reported in the literature (Hess, 1988). Some of these drugs are listed in Table 2.

ANA induced by antituberculous agents have been reported by a number of workers. Various infections can also induce ANA production, and will be discussed below.

1.3.1 Anti-DNA Antibodies

Double-stranded DNA is not immunogenic in normal individuals despite repeated injections, and antibodies are only produced in experimental situations in conjunction with an immunogenic protein.

Antibodies to ds-DNA (Figures 6 and 7) were first reported by Cepelini et al. and Holman and Kunkel in 1957. Anti ds-DNA antibodies are found in 50 to 80% of untreated SLE patients. They may also be present in Sjögren's syndrome and in chronic active hepatitis.

Antibodies to DNA are classified into those reacting against double-stranded or native molecules of DNA and antibodies to denatured or single-stranded DNA.

Circulating antibodies to DNA may be found in both IgG or IgM subclasses. IgG antibodies are more likely to be associated with clinically active SLE with nephritis than are IgM antibodies. Factors thought to influence the production and

Table 2 Pharmacological agents associated with drug-induced Lupus

Drugs with definitive association with SLE	Drugs with probable association with SLE	Drugs with possible association with SLE
Hydralazine	Anticonvulsant agents	Para-aminosalicylic acid (PAS)
Procainamide	Antithyroid drugs	Oestrogens
Isoniazid	Penacillamine	Gold salts
Methyldopa	Sulphosalazine	Penicillin
Chlorpromazine	β Blockers	Griseofulvin
Quinidine	Lithium	Reserpine
		Tetracycline

(Hess, 1988)



Figure 6 Positive anti ds-DNA using the Crithidia luciliae method

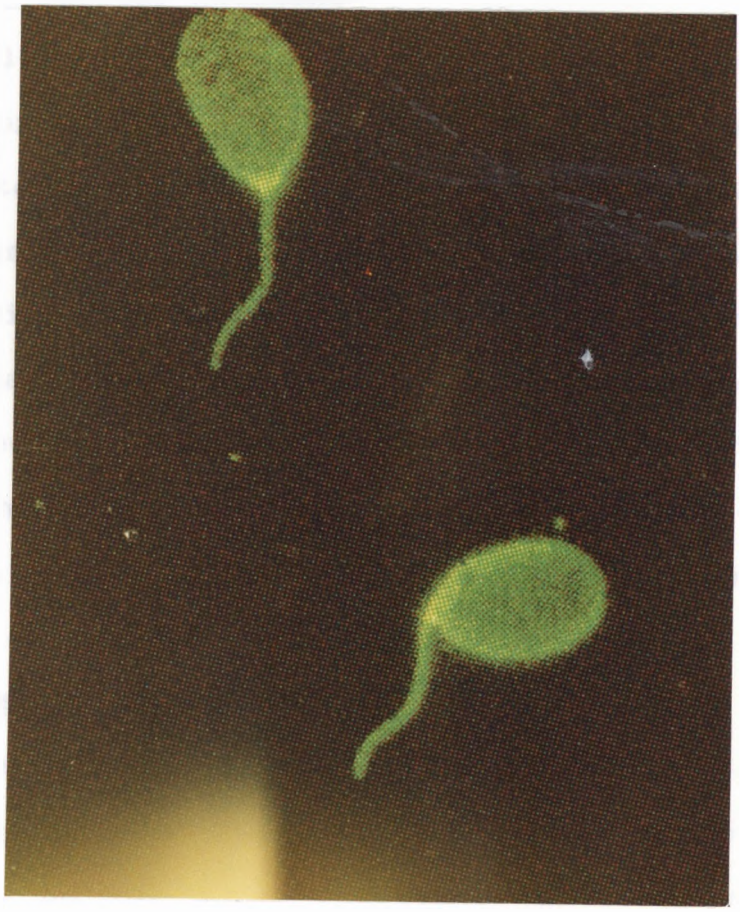


Figure 7 Negative anti ds-DNA using the Crithidia luciliae method

persistence of antibodies to DNA include genetic control of the immune response, environmental stimuli such as viruses, bacteria, drugs and ultraviolet light, the sex hormone status of the host, availability of DNA antigen, and lastly clearance of immune-complexes by the reticuloendothelial system.

1.3.2 Extractable Nuclear Antigens

Extractable nuclear antigens (ENA), prepared by the extraction of soluble proteins from cells into buffered saline, were first reported by Sharp et al. in 1972. The ENA contains RNA and a protein with two different reactive components, the ribonuclearprotein (RNP) fraction and the Sm (named after a patient Smith) fraction. Antibodies to these two components can be differentiated by digesting the antigen with ribonuclease which removes the RNP but not the Sm. Other antigens with associations with autoimmune diseases have also been identified in the ENA.

1.3.2.1 Antibodies to RNP and Sm

High titres of antibodies to RNP are associated with mixed connective tissue disease but do also occur in SLE. Antibodies to the Sm (or RNase resistant) fraction were first reported by Northway and Tan (1972), and are fairly specific for SLE, but are only present in 30% of cases.

1.3.2.2 Antibody to SS-A (Ro) and SS-B (La)

Anti-Ro (named after a patient) was originally detected by Reichlin and Mattioli (1972) as an anticytoplasmic component antibody in patients with SLE who were ANA negative. The same

author subsequently described another antibody in SLE patients with different reactivity to cytoplasmic antigens, and this antibody was called anti-La (Mattioli and Reichlin, 1974).

Alspaugh and Tan, (1975) independently detected anti SS-A and SS-B autoantibodies in patients with Sjögren's syndrome. Further investigations demonstrated that anti-SS-A and anti-Ro and anti-SS-B and anti-La were the same autoantibodies (Alspaugh and Maddison, 1979).

Although the antigenic material for anti-SS-A and anti-SS-B was originally thought to be cytoplasmic, it is now known that these antigens are present in both the cytoplasm and nuclei.

The clinical syndrome of congenital heart block in new born infants of mothers with positive anti-SS-A antibodies, with or without SLE, has recently been described (Franco et al. 1981; Kephart et al. 1981). Table 3 summarises the frequency of autoantibodies in the clinical syndromes of connective tissue disorders.

1.4 RHEUMATOID FACTORS

Rheumatoid factors (RF) are antibodies specific to antigenic determinants on the Fc fragment of human or animal immunoglobulin G. They circulate in plasma as IgG, IgA, IgM or IgE RF.

They were originally described by Waaler (1940) and Rose et al. (1948), who noted that these antibodies agglutinated

Table 3 Frequency of Positive Serologic Reactions of Antinuclear Antibodies in Various Clinical Autoimmune Disorders

Disorder	FANA	Anti-ds DNA	Anti-Sm	Anti-RNP	Anti-SSA/ Ro	Anti-SSB/ La	Anti-histone	Anti-centromere
Idiopathic SLE	>95%	50-80%	25-40%	26-45%	30-40%	0-15%	25-60%	-
Drug-induced SLE	>95%	Rare	Rare	-	-	-	90%	-
Mixed connective tissue disease	99%	Rare	Rare	100%	Rare	0-20%	-	-
Rheumatoid arthritis	20-50%	3-5%	Rare	10%	5-7%	-	20%	-
Sjögrens syndrome	20-50%	0-29%	Rare	Rare	20-70%	33-60%	-	-
Scleroderma	30-50%	<5%	Rare	22%	0-7%	Rare	-	(CREST) 80-90%
Polymyositis	20-30%	Rare	Rare	Rare	0-9%	Rare	-	-

(Tan, 1983)

sheep erythrocytes sensitised with rabbit antibodies to erythrocytes. As this antibody occurred in the serum of a large proportion of rheumatoid arthritis (RA) patients, they named it 'Rheumatoid Factor'. However, RF is not pathognomonic of RA; this autoantibody may be present in a number of rheumatic and non-rheumatological diseases, the latter being listed in Table 4.

Chronic antigenic stimulation can lead to the production of RF. Van Snick and Coulie (1983) demonstrated the production of RF in mice in response to secondary immunisation, but this response was absent in the primary immunisation process. Chronic immunisation with bacterial antigens can induce RF in rabbits (Bokisch et al. 1973) and murine lymphocytes can be induced to secrete rheumatoid factor after stimulation with bacterial lipopolysaccharides (Dresser, 1978; Izui et al. 1979).

Clinically RF may be produced in patients with chronic infection. Williams and Kunkel (1962), and Carson and Lawrance (1978), have shown that a high proportion of patients with infective endocarditis have a positive RF.

The function and pathogenic role of RF in rheumatological and non-rheumatological conditions has not been well established. A number of studies have suggested a possible protective role for RF. Van Snick et al. (1978) showed that RF protected animals against immune-complexes in chronic infections. Bolton et al. (1982) confirmed that RF reduced the total complement binding to immune complexes, thereby preventing the development of glomerulonephritis. Morgan et al. (1979)

Table 4 Diseases commonly associated with Rheumatoid Factor

Rheumatic diseases: rheumatoid arthritis, systemic lupus erythematosus, scleroderma, mixed connective tissue disease, Sjögren's syndrome.
Acute viral infections: mononucleosis syndromes, hepatitis, influenza, and many others; post-vaccination (may yield falsely elevated titres of antiviral antibodies.
Parasitic infections: trypanosomiasis, kala-azar, malaria, schistosomiasis, filariasis.
Chronic inflammatory diseases: tuberculosis, leprosy, yaws, syphilis, brucellosis, infective endocarditis, salmonellosis.
Neoplasms: after irradiation or chemotherapy.
Other hyperglobulinaemic states: hypergammaglobulinaemic purpura, cryoglobulinaemia, chronic liver disease, sarcoid, other chronic pulmonary diseases.

(Carson, 1985)

postulated that RF could clear the tissues of idiotype-anti-idiotype immune-complexes. Contrasting work hints at a pathogenic role for RF. Tanimoto et al. (1975) demonstrated that the classical complement pathway could be activated by IgM-RF. Patients with leprosy, TB and infective endocarditis exhibiting high titres of RF were shown to have more severe disease than those lacking RF (Reyes et al. 1983).

1.5 MYCOBACTERIUM TUBERCULOSIS AND AUTOIMMUNITY

Evidence to support the notion that Mycobacterium tuberculosis (MTB) may be involved in the development of autoimmune reactions comes from the Adjuvant Arthritis (AA) model; an experimental model of autoimmune disease that can be induced in susceptible strains of rats by a simple injection of complete Freund's adjuvant containing killed MTB in oil (Pearson, 1956; Pearson,1964). These rats develop a pathological picture similar to RA. A similar clinical reaction has been described in a proportion of patients with malignant diseases who are receiving immunotherapy with BCG (Torisu et al. 1978-79). These patients developed arthritis which was said to be similar to AA.

Lindqvist et al. (1970) reported a high incidence of autoantibodies in the sera of 60 patients with pulmonary tuberculosis (PTB): 46,6% of these patients had a positive ANA, 40% had a positive RF, and 21.1% demonstrated false-positive biological reactions for syphilis. These workers concluded that

there is a high prevalence of autoantibodies in TB (Lindqvist et al. 1970). However, most of these patients had been on treatment with INH.

In a 5 year follow-up study, Maisch et al. (1982) analysed the presence of autoantibodies in 20 patients with untreated PTB, 10 with chronic constrictive pericarditis, 12 with tuberculous pericardial effusions and 10 patients with viral pericarditis due to Coxsackie B virus. ANA was positive in 33% of the tuberculous pericarditis group and in 10% of those patients with chronic constrictive pericarditis. In this study a number of other autoantibodies, ie. antisarcolemal, antimyolemal, antiactin, antimyosin and antimitochondrial antibodies were also found, being more frequent in the tuberculous pericarditis group. The titres of ANA were not recorded in this study (Maish et al. 1982).

Holoshitz et al. in 1986 demonstrated that T lymphocytes of RA patients had augmented reactivity to a fraction of Mycobacteria that is cross-reactive with cartilage.
These authors studied 3 groups of RA patients (Holoshitz et al. 1986). The first group had disease of one year's duration, the second group 1-10 years duration and the third group had disease of more than 10 years duration. The T lymphocyte augmented reactivity was present in the synovial fluid of those patients with 1 year's duration of disease, and in synovial fluid and peripheral blood in the patients with 1-10 years disease duration, but was absent in the group of patients with RA of more than 10 years duration. They concluded that MTB plays an

important role in the pathogenesis of RA. Furthermore, Ottenhof et al. (1986) postulated that HLA DR4 regulation of the immune response to MTB may be important in the pathogenesis of RA. Their study group consisted of 86 leprosy patients injected intradermally with four different mycobacterial antigens. There was a statistically significant hyperreaction in response to MTB specific antigens in HLA DR4 patients when compared to other HLA subtypes and the other three mycobacterial antigens.

A cross-reactivity between mycobacterial antigens and the proteoglycan molecule (possibly core protein of chondroitin sulphate) of cartilage was demonstrated by van Eden et al. in 1985.

Monoclonal antibodies to DNA have been synthesised from autoimmunised mice and from patients with SLE. Studies with these monoclonals have suggested that some of them are also reactive to the phospholipid component found in the cell membrane or to the bacterial constituents (Eilat et al. 1986). Thus it has been postulated that the immunogenic stimulus for the production of anti-DNA antibodies may be the cell membrane or bacterial components rather than the DNA molecule itself.

Sela et al. (1987) studied the sera of 51 patients with active, untreated PTB. These workers found that 46% of the patients reacted to the 16:6 anti-DNA idiotype monoclonal antibody as compared to only 4% of the matched controls. The significance of this finding is not clear, but provides further evidence of a possible association between autoimmunity and mycobacterial disease. This series of patients also

demonstrated increased activity against a variety of antigens such as single-stranded DNA, ds-DNA, polynucleotides and anticardiolipins.

1.6 ANTIPHOSPHOLIPID AND ANTICARDIOLIPIN ANTIBODIES : CLINICAL SYNDROMES

Antiphospholipid antibodies have been detected in patients with infections and connective tissue diseases since the introduction of the Wasserman reaction (Wasserman et al. 1906). However, the term 'cardiolipin' was introduced by Pangborn in 1941. He showed that the antigen isolated from alcoholic extracts of heart and muscle was a phospholipid, hence the name cardiolipin.

The molecular structure of cardiolipin is a diacyl glycerol bound to a phosphodiester (Figure 8). The main compounds identified in the phospholipid group are phosphotidic acid, phosphatidyl inositol, cardiolipin and phosphatidyl serine, all sharing the same basic structure.

With the introduction of the specific Treponema pallidum immobilisation test in the late 1940s (Moore and Mohr, 1951), it became apparent that non-treponemal conditions could cause false positive Wasserman reactions. Furthermore, these patients had no clinical evidence of syphilis infection. Moore and Mohr (1951) introduced the concept of acute and chronic biological false-positive reactions (BFP). These acute biological false-positive reactions occur during or shortly after a wide

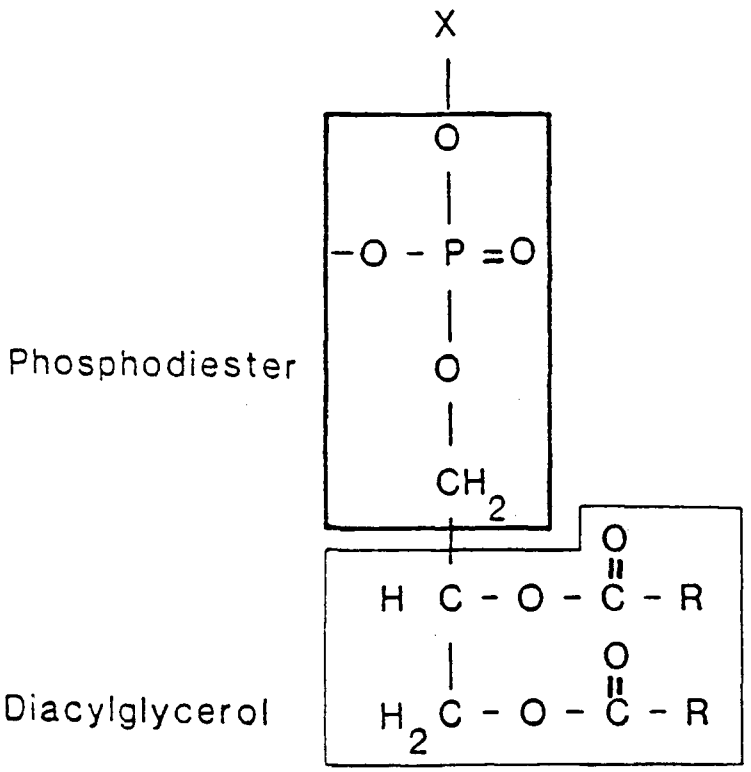


Figure 8 Basic structure of a phospholipid, consisting of a diacylglycerol group, a phosphodiester group, and (X) a substituted group characteristic of the specific phospholipid. The polar region of the molecule, consisting of two phosphodiester groups and a τ-hydroxyl, is the presumed epitope of the molecule.

variety of unrelated non-syphilis infections, and disappear in less than 6 months. The chronic biological false-positive reactions are characterised by the fact that they have been positive for years or even a life-time. These workers identified leprosy and SLE as the most important causes of chronic BFP reaction (Moore and Mohr, 1951). The incidence of BFP in non-syphilitic conditions is shown in Table 5.

In 1951 Haserick and Long established a relationship between chronic BFP reaction and SLE (Appendix 1). Their study comprised 5 female patients, with a BFP reaction occurring between one and 7 years before the first symptoms of SLE (Haserick and Long, 1951). The same observations were noted by Moore and Lutz in 1955. False positive reactions are now included in the criteria for the diagnosis of SLE (Appendix 1). Thus, where the prevalence of syphilis is high, confusion in the diagnosis may arise.

Parallel to the above observations, Conley and Hartman (1952) reported a circulating anticoagulant present in the sera of patients with SLE. They established that this anticoagulant was responsible for the prolonged prothrombin time that occurs in vitro.

Laurell and Nilsson (1957) demonstrated the association of the BFP reaction, the presence of a circulating anticoagulant and the anticardiolipin specificity of the antibody. These workers presented two cases of collagen vascular disease with a haemorrhagic diathesis which in retrospect was probably due to thrombocytopaenia, as paradoxically the circulating

Table 5 Approximate Incidence of Biologically False Positive Reactions in Various Nonsyphilitic Conditions

Infections			
	Approximate Incidence of BFP* Reactions		Approximate Incidence of BFP* Reactions
Disease	%	Disease	%
Bacterial		Rickettsial	
Leprosy	60	Typhus	20
Tuberculosis, advanced	3-5		
Pneumonia, pneumococcal	2-5	Protozoal	
Infective endocarditis	5	Trypanosomiasis	10
Chaneroid	5		
Scarlatina	5	Viral	
		Vaccinia	20
Spirochetal		Pneumonia, "atypical"	20
Leptospirosis	10	Measles	5
Relapsing fever	30	Chickenpox	5
Rat-bite fever	20	Lymphogranuloma venereum	20
		Infections hepatitis	10
Plasmodial		Infectious mononucleosis	20
Malaria	100	Common cold	? low

Noninfectious Diseases or Conditions	Approximate Incidence of BFP* Reactions
Lupus erythematosus *disseminated or discoid	20
Rheumatoid arthritis	5
Blood loss, repeated (as in multiple donations for transfusion)	? low
Pregnancy	? low

*BFP = Biologically false positive

(Moore and Mohr, 1952)

anticoagulant is associated with thrombotic episodes. This circulating anticoagulant was termed the 'Lupus anticoagulant' (LA), being primarily detected in patients with SLE. However, the 'LA' has also been detected in other conditions including autoimmune disorders, infectious diseases (Schleider et al. 1976), malignancies (Mueh et al. 1980) and drug-induced disorders (Canoso and Lise 1982).

1.6.1 Clinical Syndromes Associated with Anticardiolipin Antibodies

The 'Anticardiolipin Syndrome' has recently been described as consisting mainly of recurrent episodes of thrombosis, recurrent abortions and thrombocytopaenia with or without associated bleeding disorders (Hughes et al. 1986). The hallmark of this clinical syndrome is the detection of anticardiolipin antibodies in the serum of patients.

While the syndrome was originally described in the subgroup of patients with SLE, anticardiolipin antibodies have also been detected in patients who have no clinical evidence of SLE. This group of patients manifested venous thromboses, eg. recurrent deep vein, axillary, inferior vena cava and retinal vein thrombosis, and arterial thromboses, ie. cerebrovascular accidents, peripheral arterial gangrene, coronary thrombosis and retinal artery thrombosis.

Hamsten et al. (1986) studied 96 survivors of acute myocardial infarction, all under 45 years of age. In a follow-up period of 36 months after the acute event 13 patients (21%) were

found to have raised levels of anticardiolipin antibodies. In the follow-up period of 36 to 64 months after the first myocardial infarction, 8 of these 13 patients developed additional cardiovascular complications, including arterial occlusions, new myocardial infarctions, pulmonary emboli and deep vein thromboses. These workers concluded that anticardiolipin antibodies are of high risk for recurrent cardiovascular events.

Obstetric syndromes, such as recurrent intra-uterine death, have been reported by several groups of workers. Lubbe et al. (1983) reported 6 patients with episodes of recurrent abortion. Four of these 6 patients had clinical features of SLE, as classified by the American Rheumatism Association in 1982, (Appendix 1) and ANA was positive in all 6 patients. After treatment with prednisone and aspirin, and suppression of the lupus anticoagulant activity, all 6 patients gave birth to live infants. Carreras and Vermylen in 1982 reported on a 31 year old woman with a history of recurrent arterial thromboses and abortions in whom the 'LA' was identified. In addition, this patient had decreased levels of prostacyclin in the sera. These workers postulated that anticardiolipin antibodies could interfere with the production or release of PGI_2 by the vessel wall, thus increasing the tendency for thrombosis.

The third important clinical feature of this syndrome is thrombocytopaenia that is often acute in nature. Harris et al. (1985) studied 96 patients diagnosed as having Autoimmune Idiopathic Thrombocytopaenic Purpura, and found anticardiolipin

antibodies in 30 of the 96 patients studied (31.2%). They postulated that anticardiolipin antibodies bind to platelets, thus increasing the uptake by the reticuloendothelial system, leading to peripheral destruction and sequestration.

Finally, other features occasionally associated with this syndrome include Coombs positivity, migraine, chorea, epilepsy, chronic leg ulcers, and possible progressive dementia due to repeated cerebrovascular thrombosis (Hughes et al. 1986).

Controversial data on the role of anticardiolipin antibodies in SLE have been reported. Lockshin et al. (1985) conducted a prospective study on 19 pregnant women with SLE, and concluded that anticardiolipin antibody was the most sensitive assay in predicting foetal distress or death in pregnant women with SLE. However, Sturfelt et al. (1987) prospectively studied a group of 59 patients with SLE in whom they compared the occurrence of various clinical parameters in ACA positive and negative patients. They found no significant correlation between increased amounts of anticardiolipin antibodies and clinical symptoms such as thrombocytopaenia or thrombosis.

CHAPTER 2

PATIENTS AND METHODS

2.1 INFORMED CONSENT

Prior to undertaking the study, the protocol was submitted to and approved by the Committee for Research on Human Subjects of the University of the Witwatersrand, Johannesburg. Informed consent was obtained from all the patients participating in the study.

2.2 PATIENTS

A clinical history was obtained and patients were examined for associated disease. In the protocol it was stipulated that patients with clinical evidence of connective tissue diseases were to be excluded from the study; however, no patient demonstrated such features.

Four groups of patients were included in the study. Table 6 summarises the demographic data.

2.2.1 Group 1 - Leprosy

Forty-one consecutive black in-patients from Westford Hospital, Pretoria, were classified according to the Ridley and Jopling Classification (1966) (Table 7) into the following disease subclasses:

Table 6 Clinical Characteristics of the Study Population

	Leprosy	Untreated TB	Treated TB	Controls
Number of Patients	41	49	73	100
Male:Female Ratio	21:20	32:17	40:33	50:50
Mean Age (±SD)	44.8 yrs ±13.9	35.1 yrs ±12.4	37.3 yrs ±12.4	35.7 yrs ±8.7
Mean Disease Duration (±SD)	12.6 yrs ±12.4	1.9 months ±1.6	7 months ±7	-
Mean Treatment Duration (±SD)	12.6 yrs ±12.4	-	3.6 months ±0.2	-

Table 7 Ridley & Jopling Classification of Leprosy

Five Group Classification	TT	BT	BB	BL	LL
Lepromin reaction	3+	1+	-	-	-
Immunological Stability	++	±	-	±	-
Borderline reactions	-	+	++	+	-
Erythema nodosum leprosum (ENL)	-	-	-	-	+
Bacilli in nose	-	-	-	+	++
Bacilli in granuloma	-	1-3+	3-4+	4-5+	5-6+
Epithelioid cells	+	+	+	-	-
Langerhans giant cells	+	+++	++	-	-
Globi	-	-	-	-	+
Foam cells	-	-	-	+	+++
Lymphocytes	+++	++	+	3/1+	±
Erosion of epidermis	+	-	-	-	-
Infiltration of sub-epidermis zone	+	+/-	-	-	-
Nerve destruction (skin)	++	++	+	±	-

(Ridley and Jopling, 1966)

TT = Polar tuberculoid; BT = Borderline tuberculoid; BB = Borderline; BL = Borderline lepromatous; LL = Polar lepromatous

Polar tuberculoid	2
Borderline tuberculoid	7
Borderline	4
Borderline lepromatous	10
Polar lepromatous	18
Total	41

Complications

Erythema nodosum leprosum	5
Reversal reaction	1

Most patients required treatment with Clofasemide, Dapsone or Rifampicin. No patient had any associated medical disease.

2.2.2 Group 2 - Untreated Pulmonary Tuberculosis

Forty-nine consecutive black pulmonary TB patients presenting to the Hillbrow and Baragwanath Hospitals with a clinical history, physical examination and radiological features compatible with pulmonary TB were studied. All patients had TB by sputum examination.

2.2.3 Group 3 - Treated Pulmonary Tuberculosis

This group comprised seventy-three black PTB in-patients on treatment at the Rietfontein Hospital, Johannesburg. In addition to having the clinical and radiological features of PTB, all patients had bacteriological confirmation by examination of the sputum at the time of diagnosis. Sixty-five patients were on INH and other drugs. Eight patients were not treated with INH.

2.2.4 Group 4

One hundred healthy blood donors from the Highveld Blood Transfusion Service, Johannesburg, were selected to provide an age distribution similar to the TB groups.

2.3 METHODS:

All laboratory tests were performed at the Serology Laboratory, Department of Immunology, South African Institute for Medical Research, Johannesburg.

These tests are described in the sections below.

2.3.1 ANA

2.3.1.1 Principle

The indirect immunofluorescent test is widely considered to be the most reliable screening test for ANA. A dilution of human serum is placed on a cryostat section of rat liver and kidney which leads to attachment of immunoglobulin molecules with antinuclear specificity (cell culture has also been utilised as a substrate - eg. Hep-2 cell line). After suitable washing, a fluorescein-tagged antihuman immunoglobulin antibody bound to the nucleus is visualised by fluorescence microscopy. When viewed through the microscope, the positive nuclei appear apple-green, reflecting the colour of the bound fluorescein-tagged antihuman immunoglobulin antibody.

2.3.1.1.1 Tissue section method

- Four micron tissue sections of rat kidney and liver were cut and placed on slides. They were then frozen to be used later. When ready to test, the tissue sections were removed from deep freeze and the slide air dried for 5 minutes. The slide was fixed in acetone for 2 minutes, then was ready for use.

2.3.1.1.2 HEP-2 cell line method

Commercially available slides (Kalestad Laboratories, Federal Republic of Germany) of a cultured cell line are used in this method instead of rat liver and kidney sections.

2.3.1.2 Detection of antinuclear antibodies

2.3.1.2.1 Screening technique for both Tissue Section and Hep-2 cell line methods:

- The serum was heated for 30 minutes at 56°C prior to use. A microtitre plate was set out with serial dilutions of serum ranging from 1:5 to 1:640. Fifty microlitres of 1:20 dilution of the patient's serum was pipetted onto the antigen spot (tissue section or cell line) on the slide, making sure to spread sera all over the antigen spot. The slide was placed in a humidifier box and left to react for 30 minutes. The slide was then carefully rinsed with PBS. The slide was washed twice in PBS with a magnetic stirrer for 10 minutes each time and then rinsed in distilled water.
- Fifty microlitres of anti-human immunoglobulin antibody (fluorescent isothiocyanate (FITC) conjugated) (1:40 for HEP-2 Cell Line Method and 1:20 for the Tissue Section Method) was added to each antigen spot (making sure that all antigen spot is covered by conjugate). The conjugate was left for 30 minutes in a humidifier box to react. The slides were washed twice in PBS with a magnetic stirrer for 15 minutes each time and then rinsed in distilled water.
- The slide was air dried and then mounted in buffered glycerol and covered with a cover slip. The slide was now ready to examine under ultraviolet light.

2.3.1.3 **Interpretation of the test**

- Positive and negative controls from the local laboratory were used in addition to reference sera from an external laboratory. Those sera considered to be positive were then further tested for titration.

2.3.1.4 **Titration method**

- Serum dilutions from 1:40 up to 1:640 were made up. The slides were processed by the screening technique described in section 2.3.6.2.1 previously, and examined under an ultraviolet light.

2.3.2 Detection of Antibodies to dsDNA using Crithidia Luciliae

2.3.2.1 Principle

The detection of anti-dsDNA antibodies employs the kinetoplast of the non-pathogenic haemoflagellate Crithidia luciliae as a DNA substrate. This is an indirect immunofluorescence technique for detection of antibodies specific for dsDNA. The presence of fluorescence against the kinetoplast indicates the presence of anti ds-DNA antibodies.

2.3.2.2 Method

2.3.2.2.1 Preparation of the Crithidia slides

- 0,2ml aliquots of Crithidia luciliae suspension is transferred weekly into 5ml quantities of fresh Crithidia luciliae culture medium. This technique assures that the organism grows in the logarithmic phase, with maximal development of the various organelles. The cultures were always checked for motility and bacterial growth.
- A 5ml bottle of actively growing Crithidia luciliae culture was dispensed into 1ml aliquots.
- Subsequently the aliquots were centrifuged slowly for 20 minutes (to ensure minimal damage of the organism).
- The supernatant was decanted and an equal volume of distilled water containing 0,1% bovine albumin was added (to inflate the Crithidia luciliae).

- Using a Pasteur pipette, a drop of the Crithidia luciliae suspension was sucked up gently and placed in wells; the excess was immediately sucked off, ensuring a smear with an optimal quantity of Crithidia luciliae organism.
- The slides were fixed in 96% methanol for 10 minutes and then stored at -20°C until used.

2.3.2.2.2 <u>Qualitative serum method</u>

- A microtitre plate was set up with the following serum dilutions: 1:5; 1:10; 1:20; 1:40; 1:80; 1:160: 1:320.
- Crithidia luciliae slides were removed from -20°C freezer and dried in front of an electric fan for 15 minutes.
- The neat serum and the 1:5 dilution were placed on the microscope slide. The slides were then incubated at room temperature in a moist chamber for 30 minutes and washed twice for 10 minutes in PBS with a magnetic stirrer, and rinsed in distilled water. After washing the slides were carefully blotted dry.
- A drop of 1:40 FITC conjugated polyvalent antihuman immunoglobulin antiserum, previously titred by checker board titration, was placed on each circle of the slide and then incubated in a moist chamber for 30 minutes.
- The slides were gently washed in PBS for 30 minutes (procedure as described above), carefully blotted dry, mounted in buffered glycerol and covered with a cover slip. The slides were then examined using an ultraviolet microscope.

2.3.2.2.3 Interpretation of the test

- Slides were examined using the 50x oil objective of an ultraviolet microscope. A positive assessment result was made when fluorescence on a number of organisms from different fields, and not just a single organism was observed, as inadequate mixing of serum or FITC antihuman globulin with residual PBS on the slide could produce a slight variation in staining from field to field.
- Three types of fluorescence were noted.

(i) "Kinetoplast"

This type appeared as a very discreetly outlined fluorescent ring or doughnut appearance lying against the cytoplasmic membrane about equidistant from the opposing nuclear and flagellar ends of the organism. This type of fluorescence is specific for dsDNA.

(ii) "Nucleolar"

This type, which occurred as a faint fluorescence in the anterior end of the organism, was much larger in size than the kinetoplast pattern, and not specific for anti ds-DNA.

(iii) "Flagellar Base"

The last pattern of fluorescence appeared as a bright dot where the flagellum attaches to the body of the organism. Its significance is unclear but is not related to anti-dsDNA antibodies.

2.3.3 Poly-ENA Extractable Nuclear Antigen Assay for Detection of Antibody to SS-A (Ro) and SS-B (La)

2.3.3.1 Principle

The technique used was the Ouchterlony double immunodiffusion plate (Zeus Laboratories, New Jersey) which employs extracts of non human mammalian thymus and spleen (the source of Ro and La antigens) with a panel of standardised reference sera against these antigens. Identification of a specific precipitin line can be made by observing line fusion.

2.3.3.2 Method

- The centre well of the plate was filled with 100 ,ul of Poly-ENA SS (commercial product that contains SSA(Ro) and SSB(La) antigens).
- Well number 1 was filled with 45 ,ul of SSA/SSB positive control serum (Product number 2609 of the commercial kit).
- Well number 2 was filled with 45,ul of the negative control serum.
- Wells numbers 3 to 6 were filled with 45,ul of undiluted test sera.
- The plate was incubated in a refrigerator and observed at 24 and 48 hours for precipitin lines.

2.3.3.3 Interpretation of results

- As none of the test sera demonstrated precipitin lines during the screening procedure, further analysis of identification was not performed. It was concluded that the anti-SSA and anti-SSB antibodies were not present in the individuals tested.

2.3.4 Detection of Antibody to Nuclear Ribonuclear Protein and Nuclear Acidic Protein SM

2.3.4.1 Principle

The detection of these two antibodies is done by counter-immunoelectrophoresis. The nuclear ribonuclear and the nuclear acidic protein antigens are electrophoresed from a cathodal (-) well for a reaction with specific antibody which has moved from an anodal well (+).

SM is saline soluble and its antigenicity is not destroyed by RNase. However, while RNP is also soluble, it antigenicity is destroyed by RNase.

2.3.4.2 Preparation of a gel plate

- Ten millilitres of agar (composed of 1% agar and equal quantities of diethylbarbiturate-acetate buffer and distilled water) were pipetted into a clean Kodak lantern slide.
- This gel plate was placed in a humid box and put into a refrigerator for 30 minutes to allow the plate to harden. Wells were punched as needed.

2.3.4.3 The extractable nuclear antigens (ENA)

- The ENA consisting of the above 2 antigens amongst others, was prepared at the Serology Laboratory of the South African Institute for Medical Research; 60mg rabbit thymus powder was mixed with 1.0 ml PBS and left to stir slowly overnight at 4°C. The following morning it was

centrifuged at 5000 rpm for 10 minutes at the same temperature. The supernatant was recentrifuged and this preparation, made up to 10-12mg protein per millilitre, was used as the ENA.

- The ENA antigen was used for the detection of anti-RNP antibodies. For the detection of anti-Sm antibodies the Sm had to be extracted from the ENA antigen by RNase treatment.

2.3.4.4 Method

- 1:2 diluted serum was heated for 30 minutes at 56°C.
- The gel plate was placed in an electrophoresis bath with PBS at a pH of 8.4.
- Paper wicks were added to the side of the plate to allow electrophoresis to occur.
- The power was switched on at 5 milliamperes (in order to stop the serum from diffusing radially).
- 0,02ml of each ENA antigen was placed in the first vertical row of wells and 0,02ml of Sm antigen placed in the third vertical row.
- The serum was added to the abovementioned wells, with both a positive and negative control added to the 2nd and 4th vertical rows respectively.
- 0.01ml bromphenol blue was added to the tracking dye well.
- The current was then turned up to 10 milliamperes.
- When the tracking dye migrated 4cm the electrophoresis was stopped.

- The gel plates were placed in a humidifying chamber and incubated overnight in a refrigerator.
- The plates were soaked in 5% sodium citrate for one hour.

2.3.4.5 Interpretation of results

- Sm precipitin lines on both the Sm antigen and RNP antigen sides were visible on the positive controls. The negative control was negative.
- As there were no positive results, further titration was not performed.

2.3.5 Detection of RF with both the Rheumatec-RF Latex Test and the Rheuma-Wellcotest

RF may be demonstrated by a number of techniques, including agglutination, precipitation, complement fixation, immunofluorescence and nephelometry. With various modifications the agglutination methods are the most widely used techniques. However, if sheep red blood cells are used, it must be noted that anti-sheep heterophile antibodies may give a false positive reaction unless the sera is pre-absorbed with sheep red blood cells; this problem may be avoided by using latex particles coated with rabbit IgG (Grieble et al. 1967). Latex beads or Bentonite particles which are commercially available have been widely used. The tests are done with known standards and the results reported in international units. The exact incidence of RF in the healthy population is dependent on the method of detection, the age, social and ethnic group of the patients.

2.3.5.1 Principle

The RF reagent is an aqueous suspension of polystyrene particles coated with human gammaglobulin. If the serum containing RF is mixed with RF latex reagent, the RF reacts with the human gammaglobulin coating the polystyrene particles, and agglutination occurs.

2.3.5.2 Qualitative method

- The patient's serum and the reagent (an aqueous suspension of polystyrene particles coated with gammaglobulin and containing sodium azide as a preservative) were prewarmed to room temperature.
- Patient's serum was diluted with physiological saline in a ratio of 1:6 (Rheuma-Tec System), the Rheuma-Wellcotest System using glycine buffer in the same ratio.
- One drop (50/ul) of diluted serum was dispensed into one section of the test glass slide (provided on the commercial kit).
- Fifty microlitres of the well resuspended RF latex reagent was added to the diluted patient serum.
- Serum sample and reagent were thoroughly mixed and then spread over the entire test section with a mixing rod.
- The slide was rocked slowly to and fro for 1 minute to allow the mixture to run freely within its section.
- Agglutination was looked for and the reaction compared with controls.

2.3.5.3 Interpretation of results

- If agglutination occurred during or immediately after mixing, the presence of RF was indicated.
- If the suspension remained homogenous the test was considered negative.

2.3.5.4 Semiquantitative test

- Patient's serum was serially diluted with physiological saline. The first dilution step of 1 part serum and 9 parts saline (1:10) was prepared.
- With subsequent dilutions of the serum-saline mixture in a ratio of 1+1 (1:2) the following dilutions 1:20, 1:40, 1:80, 1:160 were obtained.
- The RF titre was the highest dilution to give an agglutination.
- Rheuma-Tec -RF Latex Test

  The correlation in IU/ml correlating with the dilution steps are listed in Table 8.

  Rheuma-Wellcotest

  Titres were expressed at the highest dilution to give agglutination.

Table 8

Dilution step	IU/ml*
1: 6	24
1: 10	40
1: 20	80
1: 40	160
1: 80	320
1:160	640

2.3.6 RPR Test

2.3.6.1 Principle

The RPR card antigen suspension is cardiolipin antigen attached to a carbon particle; it is composed of 0,03% cardiolipin, 0,020-0,022% lecithin, 0,09% cholesterol, 0,0125 M EDTA, 0,01 M $NaHPO_4$, 0,01 M $KHPO_4$, 0,1% thimerosal (preservative), 0,01875% charcoal, 10% choline chloride, W/U, and distilled water (Macro-Vue ® RPR Card Tests, BBL Microbiology Systems, Cockeysville, Md, USA). This test detects "reagins", an antibody-like substance present in serum or plasma from syphilitic subjects and in serum of patients with certain acute or chronic conditions (Table 5). When a specimen contains reagin, flocculation occurs with a coagglutination of the carbon particles of the RPR card antigen, which appear as black clumps against the white background of the plastic-coated card. This coagglutination can be read macroscopically. Non-reactive specimens appear to have an even light-grey colour.

2.3.6.2 Procedure

- The test was carried out on specially provided plastic covered card with circles.
- 50/ul of serum was placed on the circle.
- A drop of antigen was placed next to the serum (but not in contact with it). When all the test sera and the antigen suspension had been placed on the card the 2 reagents were mixed. The card was placed on a horizontal

shaker and mixed for 8 minutes at 95-110 rpm. Positive sera show clumping of carbon charged particles with varying intensity.

- No reactive sera indicates slight roughness or no clumping.

2.3.7 Fluorescent Treponemal Antibody Absorption Test (FTA- Abs)

2.3.7.1 Principle

This is an indirect immunofluorescent antibody test using fixed Treponema pallidum harvested from rabbit testes as the antigen. The test was performed with a commercially available kit (Wellcome Diagnostics, Dartford, England). The patient's serum is first absorbed with non-pathogenic treponemal antigen (absorption medium) to remove cross-reacting antibody which may have been raised against saprophytic treponemes of the oral cavity or genital tract. The test has the disadvantage of being standardised at one serum dilution (1:5), and like most other immunofluorescent tests, its interpretation is subjective.

2.3.7.2 Method

- Patient serum was heated at 56°C for 30 minutes. The slides were prepared as follows:
- Ten microlitres of treponema pallidum was spread over a multispot coated slide. The slide was air dried and then fixed in 10% methanol in distilled water for 5 minutes and allowed to dry (for fixation of the antigen to the slide). The slide was now ready for use.

2.3.7.3 Absorption test

- For absorption of non-specific antibodies the test serum was diluted 1:5 in absorption medium (1+4 parts) and incubated for 20 minutes at room temperature.
- Negative, positives and non-specific control serum samples were diluted 1:5 in absorption medium.
- Ten microlitres of the diluted and incubated samples, and the control serum was applied to the reaction zones of the prepared slides.
- It was incubated for 30 minutes at 37°C in a moisture chamber.
- It was rinsed with a 1:20 phosphate buffer solution, and placed for 10 minutes in a cuvette with phosphate buffer of the same solution. The washing solution was changed after 5 minutes.
- The slides were placed for 5 minutes in a cuvette with distilled water.
- The reaction zones were overlaid with 10,ul of the dilutions of polyvalent anti-human globulin, fluorescein-conjugate.
- The slides were incubated for 30 minutes at 37°C in a moisture chamber.
- The slides were rinsed with phosphate buffer solution, and then placed for 30 minutes in a cuvette with phosphate buffer solution. The washing solution was changed after 5 minutes.

- The slides were placed for 5 minutes in a cuvette with distilled water.
- The slides were placed with the narrow side on absorbent paper for absorption of the excess buffer and allowed to air dry.
- A 1:10 buffered glycerin solution (1 part phosphate buffer solution, 9 parts glycerin) was applied and covered with cover glass, avoiding the entrapment of air bubbles.

2.3.7.4 Interpretation of results

- The test was evaluated in a fluorescence microscope. A negativity check was done on negative controls. The weak positive control was graded as 1+ reactive and test samples read in parallel with the grading used as the baseline. Each serum test was compared with the 1+ reactive control, and those tests which gave brighter fluorescence or were equivalent to the 1+ reactive were reported as being reactive. Those that were brighter were graded 2+, 3+ or 4+, depending on the strength of the fluorescence - compared with the strong positive control which should be 4+ reactive. Those which were definitely less bright or showed no fluorescence were considered to be non-reactive.

2.3.8 TPHA TEST

2.3.8.1 Principle

The TPHA test is a specific test to detect treponemal antibodies in syphilis. These antibodies are formed only in treponemal infections and are rarely formed in collagen disorders. A measured volume of clear, haemolysis-free, unheated patient's serum is diluted with an absorbing diluent (containing antigens that bind non-specific treponemal group antibodies) starting at a dilution of 1:5.

Treponemal-sensitised ('test') red cells are added to one well, and non-treponemal ('control') red cells are added to another. The plates are left for 2 hours.

If, at the end of the sedimentation time, the red cells are agglutinated by antibody and the agglutinate settles as a mat layer in the well, the serum is then considered to be positive. Serum is considered to be negative if, after sedimentation, the red cells remain unagglutinated and settle into a small dark red button or ring in the bottom of the well.

When a positive reaction is seen in the control well containing non-treponemal sensitised cells, this is regarded as non-specific.

2.3.8.2 Screening method

- One hundred microlitres of absorption medium (composed of an ultrasonic extract of Treponema phagedenis for absorption of group specific antibodies) was introduced into the first well, and 25 microlitres of absorption medium into each of the remaining three wells.

- Twenty five microlitres of serum was added to the first well and mixed thoroughly. Twenty five microlitres of the mixture was then transferred to the second well. This method was repeated until all 4 wells had been blended. Twenty five microlitres of the mixture was then discarded from the fourth well.
- The plate was covered and left to stand at room temperature for 20 minutes in order to absorb the group specific antibodies.
- Seventy five microlitres of control reagent (consisting of stabilised sheep erythrocytes) for control against sheep erythrocyte antibodies was added to the second well. Seventy five microlitres of Treponema pallidum IHA reagent (consisting of stabilised sheep erythrocytes sensitised with Treponema antigen) was then added into the remaining wells.
- The plate was manually shaken and covered and left to stand for 2 hours at room temperature.

2.3.8.3 Titration

- Eight wells with a dilution ranging from 1:40 to 1:2560 were set out. The highest dilution still showing a positive reaction was considered the titre.

2.3.8.4 Interpretation of the test

- The plates were observed against a white background.
- Those patterns showing agglutination uniformly dispersed over the entire bottom of the well; loose spongy button or ring with irregular border; and slightly enlarged

button or ring with light, irregular border, were considered to be positive. A sharp outlined button or small ring pattern was considered to be negative.

2.4 SETTING UP THE ANTICARDIOLIPIN ANTIBODY ASSAY

2.4.1 Enzyme-linked Immunosorbent Sandwich Assay (ELISA) for Measuring IgG and IgM Anticardiolipin Antibodies

An ELISA test, as described by Harris et al. (1987) was developed for measuring IgG and IgM anticardiolipin antibodies in conjunction with the Immunology Department, South African Institute for Medical Research.

The final ELISA conditions, described below, were decided after several experiments in order to determine the optimal antigen, serum and antibody conjugate concentrations.

The variations of assay temperature and different incubation times for each step were also studied. Standard samples from known positive and negative sera were used in a calibration system to be used on every plate. The study comprised 35 SLE patients attending the Rheumatology Unit clinics at the Hillbrow and Johannesburg Hospitals. Positive sera from patients diagnosed as having SLE in whom two had a positive "lupus anticoagulant" test, and sera from healthy patients, were evaluated by Mrs R Cooper from the Microbiology Department, Tygerberg Hospital, University of Stellenbosch. All patients fulfilled the criteria of the American Rheumatism Association of 1982 (Appendix 1) for the diagnosis of SLE. An ELISA test, identical to the one used in our study, has recently

been introduced in their laboratories using standard sera provided by Dr Harris from Hammersmith Hospital, London.

The ELISA method was prepared by mixing various proportions of 3 serum samples, one of which had a high positive for IgM and IgG ACA. A second serum sample was highly positive for IgG ACA, and the third serum sample was from pooled normal human serum.

The ACA levels of these 3 samples put together forms the full sensitive range for drawing up ELISA curves.

The term GPL and MPL units have been used. The letter "G" or "M" indicate the anticardiolipin antibody isotype (eg. IgG, IgM) against phospholipid ("PL"). One MPL unit is defined as the cardiolipin binding activity of 1/ug/ml of an affinity purified IgM ACA preparation from standard serum, and one GPL unit is defined as the cardiolipin binding activity of 1/ug/ml of an affinity purified IgG ACA preparation from standard serum.

The relationship of optical absorbence versus ACA concentrations was done by plotting the log of optical absorbence versus the log of ACA level.

A linear regression equation, as described by Harris et al. (1987), was derived, as shown below:

$$\log y = a + b \log x$$

where

y = OD reading

X = IgG or IgM ACA concentration

a = interception point

b = slope of the curve

2.4.2 Standard Curve

The 3 standard serums were run on every assay ELISA plate. The standard serums were obtained as specified above. These samples had a known ACA level.

A method was used to perform a linear regression analysis of the test by entering the test ACA values for 3 calibrators as the x entries.

The test was considered acceptable if the R^2 value was ≥ 0.95.

$$R^2 = \left[\frac{N\,Exy - Ex \cdot Ey}{N \cdot Sx \cdot Sy}\right]$$

A known positive and known negative standard sample were included in each test plate and the reaction was stopped when the known positive standard reached the expected value. Results are expressed in units calculated in the first formula above. The chart below shows accepted values of ACA expressed in units (Harris et al. 1987).

High Positive	for IgG	>100 GPL units
	for IgM	> 60 MPL units
Moderate Positive	for IgG	15-100 GPL units
	for IgM	6-60 MPL units
Low Positive	for IgG	<15 GPL units
	for IgM	< 6 MPL units
Negative	for IgG	< 5 GPL units
	for IgM	< 3 MPL units

2.5 STATISTICAL ANALYSIS

Techniques used included the Pearson's Chi square test, the Student's unpaired t test, and where appropriate Fisher's exact test (2-tail).

CHAPTER 3

RESULTS

3.1 SEROLOGY

3.1.1 Rheumatoid Factor

It can be seen from Table 9 that the rheumatoid factor prevalence and titres in the population studied were low. In the leprosy group (41 patients) one patient had a positive RF with titres of 40 IU, and in the treated TB group (73 patients) 2 patients had a positive RF with titres of 80 and 160 IU respectively. In the untreated TB group (49 patients) and the control group (100 patients), all patients were RF negative.

In addition, 43 serum samples (19 untreated TB patients and 24 treated TB patients) were randomly selected for comparative testing with the Rheuma-Wellcotest RF test. Using this technique, four of the untreated TB patients (21%) had a positive RF ranging from 1:20 to 1:40 compared with only one treated TB patient (4%) with a titre of 1:40. Because of the small sample population, this difference was not statistically significant (Yates X^2 1.53; p=0.23). However in the untreated TB patients, comparison of the two test systems (Rheuma-Wellcotest and RheumaTec RF) for RF positivity, suggested borderline statistical significance (Yates X^2 2.51; p=0.05).

3.1.2 Antinuclear Antibodies

Table 10 shows that the prevalence of ANA was relatively

Table 9

Rheumatoid Factor Results in Chronic Mycobacterial Infections in Black South African patients in comparison with healthy controls

	Leprosy	Untreated TB	Treated TB	Controls
Number of Patients	41	49	73	100
RF positive	1	0	2	0
Percentage Positive	2.4	0	2.7	0
Titre in IU	40	0	80-160	0

Table 10

ANA Results in Chronic Mycobacterial Infections in Black South African patients in comparison with healthy controls

	Leprosy	Untreated TB	Treated TB	Controls
Number of Patients	41	49	73	100
ANA positive	3	3	11**	2
Percentage Positive	7.3	6.1	15.1	2
Titres	1:80	1:80	1:40-1:640	1:160-1:320
Patterns:				
Speckled	3	1	8	2
Rim	0	0	0	0
Nucleolar	0	1	2*	0
Homogenous	0	1	3*	0

* Of the treated TB patients, 9 had a single pattern, and 2 had 2 simultaneous patterns, being Nucleolar and Homogenous

** Pearson Chi Square = 10.863 p value = 0.0125

low in the leprosy group (7,3%) and although higher than the control group (2%), this was not statistically significant. ANA positivity did not correlate with reactional states. In the TB patients however, the treated patients did have a significantly increased ANA positivity (15,1%). It was noted that the 8 treated patients who did not receive INH were all ANA negative or conversely 17% of the 65 INH treated patients were ANA positive. The table also shows the range of titres found which, although mostly low, were as high as 1:640 in one of the treated patients. Although the speckled pattern was the one most frequently seen, there was no uniformity within the groups.

ANA positivity was associated with treatment duration ($p=0.0361$), being more significant for those patients on INH ($p=0.0255$) (Tables 11 and 12).

Of interest is the fact that none of the serum samples reacted against native DNA, SS-A, SS-B, RNP or Sm antigens; nor did any of the patients with or without ANA show features of a connective tissue disease.

3.1.3 Syphilis Serology

Positive syphilis serology was surprisingly high in all groups, as summarised in Table 13. At least a quarter of the subjects had evidence of past infection and, except for the untreated TB group, between 10 and 20 percent of patients were RPR positive. The control blood donors had the highest prevalence of all the groups. All patients with a positive RPR test also had positive TPHA and FTA tests. Thus, there were no

Table 11 Overall treatment duration

	ANA present (n=11)	ANA absent (n=62)
Mean duration	4.63 months	3.45 months
SD	1.28 months	1.75 months
Unpaired t-test p value = 0.0361 Statistically significant		

Table 12 Patients on Isoniazid

	ANA present (n=11)	ANA absent (n=54)
Mean	4.63 months	3.34 months
SD	1.28 months	1.77 months
Unpaired t-test P value = 0.0255 Statistically significant		

Table 13

Syphilis Serology Results in Chronic Mycobacterial Infections in Black South African patients compared with healthy controls

	Leprosy	Untreated TB	Treated TB	Controls
Number of Patients	41	49	73	100
No. RPR Positive (%)	5 (12)	1 (2)	11 (15)	16 (16)
Titres	1:2-1:64	1:64	1:2-1:64	1:2-1:64
No. TPHA Positive (%)	13 (26.5)	10 (20.4)	22 (30)	42 (42)
Titres	1:40-1:2560	1:40-1:2560	1:40-1:2560	1:40-1:2560
No FTA Positive (%)	13 (26.5)	12 (24.4)	21 (28.7)	42 (42)
Titres	1+ - 4+	1+ - 4+	1+ - 4+	1+ - 4+

biologically false positive reactors. It is interesting to note that untreated TB patients had the lowest frequency of positivity for all 3 tests.

3.1.4 Anticardiolipin Antibodies

The ACA results in Table 14 show an increase in the ACA positivity in the leprosy group compared with controls ($p=0.0461$). These patients were all on treatment. Although the frequency of positive ACA's in the treated TB group was also increased, the numbers studied did not reach statistical significance. Furthermore, none have had any of the complications associated with ACA.

Of the total of 16 positives in all the groups, only 3 patients had both IgG and IgM ACAs; the rest were either IgG or IgM positive.

3.2 OTHER PARAMETERS

1. ACA did not correlate with disease duration in the untreated TB group.
2. ACA did not correlate with the positive RPR syphilis serology test in any of the groups
3. There was no positive correlation between ACA and ANA in any group.
4. ACA did not correlate with duration of TB treatment.
5. Age and sex did not correlate with ACA.

Table 14

Anticardiolipin Antibody Results in Chronic Mycobacterial Infections in Black South African patients compared with healthy controls

	Leprosy	Untreated TB	Treated TB	Controls
Number of Patients	41	49	73	100
Number Positive (%)	5* (12)	1 (2.4)	7** (9.5)	3 (3)
IgG ACA	3*	1	3**	3
GPL units	11-29	9	8-27	8-33
IgM ACA	3*	0	5**	1
MPL units	25-121	0	6-62	40

* Of the leprosy patients, 2 had a raised IgG ACA, 2 had a raised IgM ACA, and one had both IgG and IgM ACA

** Of the treated TB patients, 2 had a raised IgG ACA, 5 had a raised IgM ACA, and one had both IgG and IgM ACA

CHAPTER 4

DISCUSSION

4.1 AUTOANTIBODIES IN LEPROSY

Bonomo et al. (1965) were the first workers to describe autoantibodies in leprosy. Subsequently the frequent occurrence of autoantibodies in patients with leprosy has been documented in a number of studies in the past 23 years, and this work adds to the extensive literature on the subject. However, this is the first study dealing with the prevalence, pattern and titres of autoantibodies in black South African patients. Moreover, a clinical overlap exists between leprosy and diseases with autoimmune phenomena, in particular SLE. In 1967, Bonomo et al. described a 42 year old female with lupoid features and having positive RF, ANA and LE cell phenomenon.

4.1.1 Rheumatoid Factor in Leprosy

Discrepancy exists in the literature about RF in leprosy. Some investigators found up to 50% of leprosy patients to have a positive RF (Abe et al. 1967), while others have failed to detect such autoantibodies (Miller et al. 1987).

A number of factors must be considered - the type of assay utilised, genetic factors, study population, the type and severity of disease and the possible role of different treatment regimens.

Difficulty exists in assaying RF because the test has not

been standardised. IgM RFs are multivalent antibodies and effective agglutinators of antigen-coated particles, such as bentonite or latex beads which have been passively coated with human IgG. The cross-linking of these IgG coated particles by IgM RF in serum produce a flocculation reaction. In addition, technical problems have arisen because the IgG antigen with which RF reacts, particularly in chronic infections where patients are hypergammaglobulinaemic, may interfere with or cross-react with a specific detection system. Positivity may vary according to size and characteristic of the particles used.

The Rheuma-Tec ® -RF latex test utilised in this study comprises an aqueous suspension of polystyrene particles sensitised with human gammaglobulin, where the positivity in patients with mycobacterial infections was shown to be low. This is in contrast to the results obtained when using the Rheuma-Wellcotest system, where 21% of the untreated TB patients tested demonstrated a positive RF. When comparing the results of the two systems, the Rheuma-Wellcotest produced a higher positivity, as shown in a similar study conducted by Chalmers et al. (1977) in Durban in 301 black South African in-patients with non-rheumatoid conditions, where there was a positivity of 25.6% (11 of 43 TB patients). Furthermore, the positivity of RF is dependent on the technique used, as documented by other investigators (Abe et al. 1967; Dhople, 1972; Petchclai et al. 1973). From the results obtained in this study it appears that the Rheuma-Tec ® -RF system produces a low cross-reactivity in chronic infections. It should be noted that

the Rheuma-Tec ® -RF latex test is routinely used in the Serology Laboratory of the South African Institute for Medical Research. It was thus of interest to assess the positivity of this system in black patients in the local setting, where there is a high prevalence of mycobacterial infections.

Genetic determinants to be considered include:

(a) RF, like any other antibodies, have specificity for defined antigens, in particular the Gm antigens of the Fc portion of the IgG molecule. These antigens differ in individuals, and possibly these variations may alter the affinity of RF. These differences may change the positivity of RF in both healthy and diseased population groups.

Furthermore, another important point to consider is that genetic factors influence the structure and specificity of the particular autoantibody produced (Carson, 1985). Previous studies have shown that RF and monoclonal proteins with RF activity share common amino acid sequences in the heavy and light chain regions. Monoclonal IgG gammaglobulinaemia also occurs in patients with hypergammaglobulinaemia, an abnormality which is known to be present in patients with chronic infections.

(b) The histocompatability-linked immune response gene HLA DR4 which has a similar prevalence in black and white South Africans (du Toit et al. 1988), does not appear to play a role in the induction of RF in normal individuals

nor in diseases other than rheumatoid arthritis (Engleman et al. 1978). In normal blood donors who are either HLA DR4 positive or negative, no statistical differences exist in RF positivity. However, HLA DR4 positive subjects have a higher risk for seropositive RA when compared with HLA DR4 negative subjects.

Finally, Reyes et al. (1983) demonstrated that RF positivity in TB patients was dependent upon disease severity. It may be possible that patients studied by other workers had more severe disease than those patients in the present study population.

Table 15 shows that the low prevalence of RF found in this study is similar to the tuberculoid patients in the study of Shwe (1972) and the whole group from the studies of Petchclai et al. (1973) and Miller et al. (1987). Petchlai et al. (1973) suggested that the low occurrence in their study could be due to a difference in the immune response among Thai patients. Contradictory results have been obtained by Abe (1967), Saha and Mittal (1972) and Dhople (1972) who studied the prevalence of RF during and after an acute lepra reaction in 39 patients; in this study the patients were divided into 2 groups - those with acute lepra reaction with arthritis (11) and those without arthritis (28). These authors found 73% of the patients in the first group (8 of 11 patients) to have a positive RF during the acute attack compared with 32% (9 of 28) with positive RF in the second group. Their conclusion was that RF may play a role in the

Table 15 Rheumatoid Factor in Leprosy

Author	Leprosy Subtype	RA Test	Percentage
Abe et al.	Lepromatous	Waaler Rose RA Test (Hylands Laboratory) FII-LA	60,4 28,0 37,0
	Tuberculoid	Waaler Rose RA Test FII-LA	53,0 6,3 28,0
Tin Shwe	Lepromatous	Latex Agglutination (Hylands Laboratory)	25,0
	Tuberculoid		0,0
Dhople	Lepromatous with Arthritis	Latex (in-house) RA Test	73.0
	Lepromatous without Arthritis	Latex (in-house) RA Test	32,0
Ellis	Lepromatous	Latex Test (Hoechst and Hylands)	18,0
	Tuberculoid		43,0
Petchclai et al.	Lepromatous	Waaler Rose Latex (Hylands Laboratory)	13,8 3,4
	Tuberculoid	Waaler Rose Latex (Hylands Laboratory)	5,2 0,0
Miller et al.	All types	Undefined	0,0
Saha and Mittal	Lepromatous	Latex Test (Sylvania)	35,0
Rapoport et al.	All types	Latex Test (Rheumatec-RF)	2,4
Chavez-Legaspi et al.	Lepromatous	Latex Test (Behring)	18,7

The table summarises the findings of RF in different investigations by different methods.

pathogenesis of arthritis and the rheumatological manifestations that patients with acute lepra reactions usually experience. However, in contrast to the study of Dhople (1972), in the present study 5 patients had ENL, and none had a positive RF (Table 15).

ENL is a reaction that occurs mainly in lepromatous leprosy patients, most commonly during the first year of treatment. It is characterised by the presence of tender, inflamed subcutaneous nodules. More severe cases may also present with a low grade fever, lymphadenopathy and arthralgia. Other symptoms include painful neuritis, most commonly involving the ulnar, the median and lateral popliteal nerves which result in weakness and anaesthesia. Rarely, patients may have nephritis, epididymo-orchitis, iridocyclitis and large joint arthritis. The clinical picture of this syndrome is similar to that of autoimmune disorders.

It may be possible that these discrepancies exist either on the basis of the different population groups studied or on the various RF assay techniques utilised.

4.1.2 ANA in Leprosy

From Table 16 it can be seen that there was also quite a wide variation in the reported occurrence of ANA, ranging from absent or very low, as in the present study, up to the 28% reported by Masala et al. (1979). Tin Shwe, in 1972, found ANA more common in the lepromatous patients, while in Masala's

Table 16 ANA in Leprosy

Author	Leprosy Subtype	ANA Test	Percentage
Wright	Lepromatous and Tuberculoid	Indirect Immunofluorescence with IgG conjugate	4,0
		As above but IgM conjugate	23,0
Tin Shwe	Lepromatous	Indirect Immunofluorescence	17,9
	Tuberculoid		0,0
Petchclai et al.	Lepromatous	Tissue Sections	0,0
	Tuberculoid		
Masala et al.	Lepromatous	Indirect Immunofluorescence	28,0
	Tuberculoid		28,0
Miller	All types	Tissue Sections and HEP-2 Cell Line	16,0
Chavez-Legaspi et al.	Lepromatous	HEP-2 Cell Line	3,1
Rapoport et al.	All types	Tissue sections and HEP-2 cell line	2,0

The table summarises ANA.

groups it was equally as common in the tuberculoid group. Low sensitivity of the ANA tests used in the present study are unlikely to be the reason for the low incidence. The tests were used routinely in the lupus clinics, where they have, over a number of years, been found to be reliable.

4.2 AUTOANTIBODIES IN PULMONARY TUBERCULOSIS

4.2.1 Rheumatoid Factor in Pulmonary Tuberculosis

In two separate studies of RF in TB patients (Cannat and Seligmann, 1966; Lindqvist et al. 1970), a higher prevalence of RF was demonstrated. Twenty-four percent of the untreated, and 28.8% of the treated TB patients had a positive RF (Cannat and Seligmann, 1966). Lindqvist et al. (1970) demonstrated that in their study RF was positive in 40% of patients with titres ranging from 1:20 to 1:640. There was no positive RF in the untreated patients in the present study, and only a positivity rate of 2.8% in the treated TB group. As mentioned previously, the laboratory technique, and possibly genetic factors, may account for this discrepancy.

4.2.2 Antinuclear Antibodies in Pulmonary Tuberculosis

ANA may be produced by a number of drugs; however, only a small proportion of patients who develop ANA from drugs will develop clinical SLE (Alarcón-Segovia, 1969a). In the present study, examination of all patients in the population tested failed to demonstrate any clinical features of SLE. This is

borne out by evidence from the Connective Tissue Disease Clinic at Hillbrow Hospital, Johannesburg, where only one equivocal case of drug-induced lupus was found in a group of approximately 100 black SLE patients (Dr RCA Morrison, personal communication). Furthermore, it must be borne in mind that in the United States of America drug-induced lupus is a rare condition in blacks (Hess, 1988).

In the untreated TB population, only 6.1% of patients had a positive ANA test compared with 17% of the treated TB group. However, none of the serum samples reacted against ds-DNA, anti-Sm, anti-SS-A, anti SS-B or anti RNP.

The induction of ANA correlated with treatment duration and not with disease duration, suggesting that ANA was induced mainly by anti-tuberculous drugs, and in particular INH. None of the patients treated with drugs other than INH had a positive ANA test. Titres were generally low, except for one patient who had an ANA positive test of 1:640. ANA was more prevalent in males than in females, this difference not being statistically significant but noteworthy, considering the predominance of females in SLE and drug-induced SLE.

The induction of ANA by anti-tuberculous drugs was first described by Cannat and Seligmann in 1966. In their study these workers compared the incidence of ANA in 125 untreated and 75 treated TB patients. The prevalence of ANA was 4% in the untreated group and 20% in the treated group. These findings are similar to those in the present study. However, the authors (Cannat and Seligmann, 1966) did not report on titres, and did

not analyse sex, age and treatment duration in relation to the induction of ANA.

Rothfield et al. (1978) in a prospective study, also demonstrated the effect of treatment. Of 192 PTB cases, 4% had a positive ANA before treatment, and 21.6% developed ANA during treatment. The role of the duration of treatment was not analysed.

Ali in 1969 found only 4 of 80 TB patients to have low titres of ANA. Lindqvist et al. (1970) found that 46% of 60 patients had a positive ANA. However, all their patients were on treatment, and the study did not divide the patients into treated or untreated TB.

It was demonstrated in 1968 by Price-Evans that INH and hydralazine were polymorphically metabolised in humans. Perry et al. (1967; 1970) and Alarcón-Segovia et al. (1969b) demonstrated that ANA is more prevalent in patients who are phenotypically slow acetylators. The results of the present study demonstrated that ANA was more prevalent in males than in females. Alarcón-Segovia et al. (1969b) analysed the incidence of ANA in 184 TB treated patients. However, their study showed a higher prevalence of ANA in females. The same workers (Alarcón-Segovia et al. 1971) in a different study, analysed 153 TB patients receiving treatment with INH. In this group of patients, 78 were slow and 75 rapid acetylators. The overall ANA positivity was 20%. In the current study, the patterns of acetylation in black South Africans was not analysed, but it has been previously demonstrated that the prevalence of both slow and fast

acetylator phenotypes is similar in black and white South Africans, with the exception of the Kgalagadi of the Sotho/Tswana tribe who have a low frequency of slow acetylation phenotype (Jenkins, 1986). There were no Kgalagadi in the present study population.

4.3 ANTICARDIOLIPIN ANTIBODIES AND SYPHILIS SEROLOGY IN MYCOBACTERIAL INFECTIONS IN BLACK SOUTH AFRICANS

The current study demonstrates the presence of circulating ACA in sera of patients with chronic mycobacterial infections.

A statistical difference was found in ACA positivity between leprosy patients and the healthy blood donor control group. However, no correlation was found with disease subtype, disease duration, sex or age.

ACA was also raised in the treated TB group. However, when compared with the untreated TB group or the blood donor group, this difference was not statistically significant.

There were no specific patterns of IgG or IgM ACA subclasses in any of the groups studied.

The role of ACA in mycobacterial infections is not clear and remains to be elucidated.

In a study of 57 untreated pulmonary TB patients, Sela et al. (1987) found a positive IgG ACA in 23% and IgM ACA in 32% of the cases studied. Their positivity was much higher than in the study in black South African patients under review. However,

their study was not compared with controls. Table 17 summarises the findings of ACA in infections.

ACA is becoming an accepted test in monitoring disease activity in SLE patients. However, ACA was previously reported to be raised in other infectious diseases. McAdam et al. (1985) studied 260 serum samples from Papua-New-Guinean malaria patients. They found that a high percentage of patients had increased prevalence of ACA, the titres being higher in those with parasitaemia and splenomegaly.

In 1987 Sturfelt et al. found that 27% of sera from 96 randomly selected patients with streptococcal infection had high titres of ACA. A follow-up ACA was performed after one year in 6 of the positive patients, with only one still having antibodies.

Vaarala et al. (1986) demonstrated raised ACA titres in patients with ornithosis, mycoplasma, adenovirus, rubella, chicken pox and mumps. Cohen et al. (1986), Canoso et al. (1987) and Oksenhendler et al. (1987) reported raised ACA levels in patients with Acquired Immune Deficiency Syndrome (AIDS).

There is no clear evidence regarding induction of ACA in chronic mycobacterial infections. However, in an attempt to explain this serological abnormality, a number of mechanisms may be postulated.

Mycobacteria contain antigens that resemble cardiolipin (McAdam et al. 1984). Thus, it is possible that the immune system is stimulated to produce antibodies which may cross-react with the ACA.

Table 17 ACA in Infections

Author	Disease	Percentage ACA
Sela et al.	Untreated PTB	IgG 23% IgM 32%
McAdam et al.	Malaria	Present
Sturfelt et al.	Streptococcal ACA positives one year later	27% 1:6
Vaarala et al.	Ornithosis Mycoplasma Adenovirus Rubella Chicken Pox Mumps	All increased
Cohen et al.	AIDS	Increased
Canoso et al.		
Oksenhendler et al.		

Another possibility is that the infecting organism, by releasing enzymes during the infectious process, alters the chemical composition of the host tissue, transforming "self" into "non-self" antigens, thus inducing production of both autoantibodies and ACA. The destruction of mycobacteria by chemotherapeutic agents or the immune system may release new antigenic structures similar to those described above (McAdam et al. 1984), thereby stimulating the immune system in the production of ACA. Finally, the drugs used to treat the conditions may induce ACA.

The current concept regarding antiphospholipid antibodies is that of a group of antibodies which may cross-react with a number of antigens, including phosphatidic acid, phosphatidyl inositol, cardiolipin and phosphatidyl serine. However, they do not all lead to the "anticardiolipin syndrome". None of the patients in the present study with positive ACAs showed thrombotic or other associated manifestations of the syndrome.

In all groups studied, it was noted that there was a high proportion with positive syphilis serology; however there was no correlation between positive syphilis serology and ACA, as confirmed by Harris et al. (1985). As sexually transmitted diseases appear to be prevalent in both mycobacterial groups and controls, it must be assumed from the above data that this population is also at risk of contracting other venereal diseases, including AIDS.

As has been previously reported (Moore and Mohr, 1951), a high proportion of patients diagnosed as having leprosy or TB

were biological false positive reactors for Lues. However, it is striking that there were no biological false positive reactors among the black South African patients studied. Syphilitic infection produces 2 types of antibodies: the non-specific 'reaginic' antibody and the 'specific' antitreponemal antibody. The former is directed against lipoideal antigens that result from the interaction of Treponemal pallidum with the host tissue, and possibly against lipoidial antigens of the Treponema pallidum itself. The latter are antibodies against treponemal antigens (Csonka GW; Holmes and Lukehart,). The RPR test used in this study detects 'reagin', being positive in BFP reactions (Table 5) and in patients with treponemal infections such as active syphilis, yaws, and pinta. This RPR test becomes positive during the primary stage of syphilis and increases up to four-fold during the secondary stage of this disease. The RPR test becomes negative after successful treatment.

The TPHA and FTA-Abs tests are specific anti-treponemal tests with a false positivity of 1 to 2%. These tests become positive during the early stages of the disease, persisting for life. In this study more patients demonstrated positive TPHA and FTA-Abs tests compared to those with the RPR test; this suggests past treated syphilitic infection. However, in light of the high frequency of positive syphilis serology it is possible that a biological false positive reaction due to a mycobacterial infection would be masked or misinterpreted as an acute syphilitic infection. However, one would still have expected

some BFP reactors in the two-thirds of patients who had negative syphilis serology. These findings should be borne in mind when diagnosing SLE, where one criteria is a biological false positive reaction (Tan et al. 1982).

The possibility exists that ACA is induced by drugs as both the treated TB group and the leprosy group were on a number of therapeutic agents. However, there has been no documentation of this in the literature. Canoso and Lise (1982) reported an association of chlorpromazine treatment with the induction of lupus anticoagulant in 11 of 30 patients. Thrombotic manifestations were absent and ACA assay was not carried out. Further studies need to be performed for this hypothesis to be elucidated.

A small, but interesting study conducted by Colaço and Male (1985) demonstrated a strong correlation between sera of 10 SLE patients with thrombotic manifestations and 10 sera samples of luetic patients with similar matched anticardiolipin activity when tested against four phospholipid antigens - cardiolipin, phosphatidic acid, phosphatidyl ethanolamine and phosphatidyl serine. A significant difference in antiphosphatidyl ethanolamine/antiphosphatidyl serine binding ratio was found between the two groups. The authors concluded that the differences in phospholipid epitope specificity may explain the different haematological manifestations between the two diseases

and that antiphosphatidyl serine could play a role in the thrombotic diathesis seen in SLE patients. Further studies of this nature will assist in the characterisation of ACA in mycobacterial infection.

CHAPTER 5

CONCLUSION

5.1 ANTINUCLEAR ANTIBODIES AND RHEUMATOID FACTOR IN CHRONIC MYCOBACTERIAL INFECTIONS

- The treatment of TB is associated with induction of ANA.
- The ANA positivity correlates with duration of treatment and not with the duration of disease.
- Only patients that were on an INH drug combination had a positive ANA.
- Contrary to drug-induced SLE, ANA was more frequent in males than in females.
- In the leprosy and in the untreated TB groups, the frequency and titres of ANA were very low.
- None of the sera reacted against ds-DNA, RNP, Sm, SS-A or SS-B antigens.
- Except for the treated TB patients, autoantibodies in chronic mycobacterial infections should not lead to confusion with connective tissue diseases in the two hospitals where this study was performed.
- Rheumatoid factor positivity and titres were low in all groups, did not correlate to treatment and is probably related to the assay used and possibly because of genetic factors in the population studied.
- There was a marked difference in RF using the different techniques.

5.2 SYPHILIS SEROLOGY AND ANTICARDIOLIPIN ANTIBODIES IN CHRONIC MYCOBACTERIAL INFECTIONS

- Leprosy is associated with the induction of ACA. However, the role of treatment and mechanism of production are still to be elucidated. The clinical relevance of ACA also needs to be clarified.
- Although ACA titres were raised in the treated TB group, this was not statistically significant; however the treated TB group had a higher incidence of ACA compared with the untreated group suggesting a possible drug related role.
- ACA positivity and titres did not correlate with the duration of disease, nor with age or sex.
- There was no specific pattern of IgG or IgM ACA subclasses.
- None of the positive ACA patients had thrombotic manifestations described in the 'antiphospholipid syndrome'.
- The prevalence of syphilis serology was high in all groups studied.
- RPR titres were low and did not correlate with ACA.
- These findings suggest that antiphospholipid antibodies react against different epitopes to those produced by syphilis, but with some cross-reaction between themselves.

- Biological false positive reactions in populations with prevalent syphilis serology are difficult to establish, and may cause confusion in the diagnosis of SLE.
- Finally, the high prevalence of syphilis serology observed in the patients studied is suggestive that this population group is at risk of contracting other sexually transmitted diseases, including AIDS.

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Appendix 1 Revised criteria for the classification of systemic lupus erythematosus

A. Malar rush. Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds.

B. Discoid rash. Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions.

C. Photosensitivity. Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation.

D. Oral ulcers. Oral or nasopharyngeal ulceration, usually painless, observed by a physician.

E. Arthritis. Nonerosive arthritis involving two or more peripheral joints, characterised by tenderness, swelling, or effusion.

F. Serositis.
1. Pleuritis. Convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion.
2. Pericarditis. Documented by ECG or rub or evidence of pericardial effusion.

G Renal Disorder
1. Persistent proteinuria greater than 0.5g/day or greater than 3+ if quantitation not performed.
2. Cellular casts. May be red cell, haemoglobin, granular, tubular, or mixed.

H. Neurologic Disorder
1. Seizures in the absence of offending drugs or known metabolic derangements (eg. uraemia ketoacidosis, or electrolyte imbalance).
2. Psychosis in the absence of offending drugs or known metabolic derangements (eg. uraemia ketoacidosis, or electrolyte imbalance).

I. Haematologic Disorder.
1. Haemolytic anaemia with reticulocytosis.
2. Leukopaenia. Less than 4000/μl total on 2 or more occasions.
3. Lymphopaenia. Less than 1500/μl on 2 or more occasions
4. Thrombocytopaenia. Less than 100,000/μl in the absence of offending drugs.

J. Immunologic Disorder.
1. Positive LE cell preparation.
2. Anti-DNA. Antibody to native DNA in abnormal titre.
3. Anti-Sm. Presence of antibody to Sm nuclear antigen.
4. False-positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilisation or fluorescent treponemal antibody absorption test.

K. Antinuclear antibody. An abnormal titre of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs, known to be associated with drug-induced lupus syndrome.

The above classification is based on 11 criteria. For the purpose of identifying patients in clinical studies, a person shall be said to have systemic lupus erythematosis if any 4 or more of the 11 criteria are present, serially or simultaneously, during any interval of observation.