Clin. exp. Immunol. (1977) **27**, 230–237.

The spectrum of human tuberculosis

L. LENZINI, P. ROTTOLI & L. ROTTOLI *Institute of Tuberculosis and Respiratory Disease, Medical School, University of Siena, Siena, Italy*

(*Received* 3 *August* 1976)

SUMMARY

Clinical, morphological and immunological studies of human tuberculosis have enabled the spectrum of the disease to be determined. We have investigated the cell-mediated immune responses by means of skin tests and leucocyte migration inhibition to PPD, and the humoral immune responses by means of immunodiffusion and haemagglutination tests.

Patients with tuberculosis can be classified into two polar groups—reactive (RR) and unreactive (UU), the former showing good cell-mediated immunity and little or no antibody formation and the latter poor cellular responses and exuberant antibody production. The intermediate forms show characteristics of the neighbouring polar groups.

The existence of a spectrum of immune response in tuberculosis, which has long been suspected, is now demonstrated.

INTRODUCTION

Recently there has been renewed interest in the immunology of chronic infectious diseases caused by intracellular parasites. With the demonstration of differences in cell-mediated immunity, a spectrum of immune responses has been shown in many diseases, such as leprosy, leishmaniasis and syphilis (Godal, 1974; Mackaness, 1968; Turk & Bryceson, 1971; WHO Scientific Group Report, 1973).

The existence of a spectrum in tuberculosis (TB) has also long been suspected. We have now been able to demonstrate with the use of *in vitro* techniques for the investigation of cell-mediated immunity that there are marked differences in immune response in this disease, which has been the object of extensive clinical research for over a hundred years (Daddi & Panà, 1947; Lefford, McGregor & Mackaness, 1973; Lurie, 1964; Rich, 1941).

The different manifestations of human tuberculosis treated with anti-tubercular drugs have been examined with conventional parameters, radiological and bacteriological; humoral and cellular immune responses have also been examined (Lenzini & Barnabè, 1969, 1973; Lenzini, Rottoli & Rottoli, 1974; Lenzini *et al.*, 1974). Morphological investigations complete the study (Lenzini & Faenzi, 1966).

MATERIALS AND METHODS

Patients. The clinical material studied comprised sixty-six individuals, thirty-one females (age range 18–80; mean 40·3) and thirty-five males (age range 12–82; mean 35) with bacteriologically or histologically confirmed tuberculosis. Cases of infection with the so-called non-classified, anonymous or atypical mycobacteria were excluded. The groups were classified on the basis of clinical and radiological data, as follows: (1) micronodular localized tuberculosis (reactive, RR); (2) nodular or micronodular localized tuberculosis with cavitation, unilateral or bilateral lymphoadenopathy, tubercular serositis (reactive intermediate, RI); (3) nodular or micronodular chronic diffuse tuberculosis with cavitation and fibrosis, tubercular lymphoadenopathy complicated by fistula formation (unreactive intermediate, UI); (4) acute miliary tuberculosis (unreactive, UU).

The definition of localized micronodular tuberculosis (1) is based on the size and discrete quality of the lesions on X-ray and their limitation to one or two segments of the lung. Nodular lesions (2) are larger in size but similarly limited in their distribution. The lesions in nodular or micronodular chronic diffuse TB (3) are characterized by the presence of one or

Correspondence: Dr L. Lenzini, Institute of Tuberculosis and Respiratory Disease, University of Siena, Siena, Italy.

more cavities, by the prolonged course of the disease and by its relative resistance to therapy. Acute miliary TB (4) is classical in its definition.

On the basis of the clinical, radiological and immunological data, the first group is defined as reactive tuberculosis (RR), the second as reactive intermediate tuberculosis (RI), the third as unreactive intermediate tuberculosis (UI) and the fourth as unreactive tuberculosis (UU) (Lenzini, 1974).

Histopathology. Pathological material was collected from surgically removed tissue at the Regional Centre of thoracic surgery of Arezzo (Ospedale Garbasso) from 1961 to 1971. We are grateful to Professor Panà for his help in collecting autopsy material. Sections were stained for routine histopathology.

Skin test. The skin tests were carried out with 5 T.U. of PPD (Sclavo) and were read after 3, 6, 24, 48, 72 and 96 hr. A transverse diameter of the infiltration-induration greater than 5 mm was considered positive; when the reactions were negative, tests were repeated with 100 T.U. PPD.

Serology. Passive haemagglutination test with sensitized tanned cells. Sheep red cells were sensitized for the passive haemagglutination test by the method of Roitt & Doniach (1972). The antigen, PPD, was prepared from a single batch by Sclavo Institute. Positive and negative sera were always used as internal controls and the plates were read after 18 hr at 4°C.

Agar immunodiffusion test. Double diffusion in 1% agar was performed on microscope slides. After 24-hr incubation at room temperature the slides were washed in veronal buffer (pH 8·2) for 4–5 days. They were then washed in 1% tannic acid solution for 5 min, in water for 2 hr, and then stained with Coomassie Blue for 5 min, and decolourized before reading.

Leucocyte migration test. 20 ml of heparinized blood was allowed to stand for about 1 hr at 37°C to permit sedimentation of the red blood cells. The supernatant was removed, centrifuged for 10 min at 700 *g* and washed three times in MEM (Eagle's Minimum Essential Medium, Burroughs Wellcome Ltd). The white blood cells in medium containing 10% foetal calf serum were drawn into capillary tubes, plugged and centrifuged at 350 *g* for 10 min. The capillary was then cut at the cell–fluid interface and the cells placed in small circular plastic chambers of 0·55 cc capacity (Brostoff, 1970), containing culture medium with PPD* enriched with 10% foetal calf serum. The chambers were sealed with cover slips and incubated at 37°C in CO_2 air environment for 20 hr. The migration areas were projected onto paper, outlined and the areas measured. The migration index (MI) was calculated as:

$$MI = \frac{\text{area with antigen}}{\text{area without antigen}} \times 100.$$

A dose range of PPD up to 500 μg/ml was used in all the patients. For simplicity, the results of a single dose are described, i.e. 125 μg/ml.

RESULTS

Histopathloogy

On the basis of morphological data, a correlation seems to exist between the cellular composition of the lesions and the host's resistance to tuberculosis. One pole of the histological spectrum shows lesions containing lymphocytes and epithelioid cells. Patients with such lesions show early reduction of the bacterial load in the sputum with treatment and localized lesions on X-ray.

The opposite pole presents lesions with polymorphonuclear leucocytes and macrophages containing mycobacteria. Undifferentiated histiocytes are also present. These lesions correlate clinically with patients who show a poor response to treatment, have persistant mycobacteria in the sputum, and also disseminated lesions. The intermediate region of this spectrum shows characteristics of both polar forms.

The full clinical and histopathological data will be reported elsewhere.

Skin test

Three types of reactions were detected (Fig. 1):

(a) typical delayed hypersensitivity reactions present at 24 hr peaking at 48 hr with strong induration persisting up to 72 or 96 hr; (b) early reactions; rapid development persisting until 24 hr chiefly with erythema and oedema, gradually decreasing and disappearing at 48 hr; (c) biphasic reactions with an early phase visible from 3 hr to 24 hr with erythema and oedema, gradually decreasing at 48 hr; persistence after 72 hr was seen when the reaction showed infiltration, induration and central necrosis.

* The PPD used was from a special bulk lot expressly prepared for our study by Sclavo Institute. Composition: proteins: 2·05 mg/ml; polysaccharides: 0·0212 mg/ml; nucleic acids: 0·052 mg/ml.

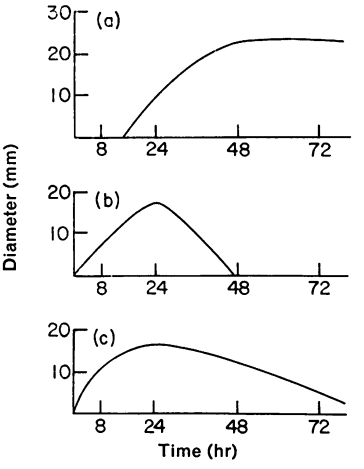


FIG. 1. The three types of skin-test seen in the patients. The delayed reaction (a) typical of the Mantoux reaction. An early reaction (b) characteristic of Jones–Mote hypersensitivity and a mixed form (c).

Serology

Haemagglutination. The range of anti-PPD antibodies in the four groups of patients is shown in Fig. 2. The controls, whether PPD skin-test positive or negative, did not have any anti-PPD antibodies as shown by sensitized tanned red cell haemagglutination. With increasing unresponsiveness clinically the percentage of patients with antibody increased as did the titre of the antibody.

Precipitin. Similar findings were shown with the precipitin tests where, in general, only patients with the unreactive form of disease (UI and UU) showed a positive result (Fig. 3).

Leucocyte migration test

The results of the leucocyte migration tests with PPD at a dose of 125 μg/ml are shown in Fig. 4. The reactive patients, i.e. RR and RI, show a range of migration inhibition similar to PPD skin test positive controls. However, the unreactive groups, UI and UU, are anergic with this test, showing migration indices similar to skin test negative controls.

The one group of special interest is UI, borderline 'unreactive'. Here, although anergic with a dose of 125 μg/ml of PPD, 23% showed a positive migration test with 250 μg PPD and 62% were positive with 500 μg/ml (Fig. 5). No other group showed this change in migration indices with different doses of antigen.

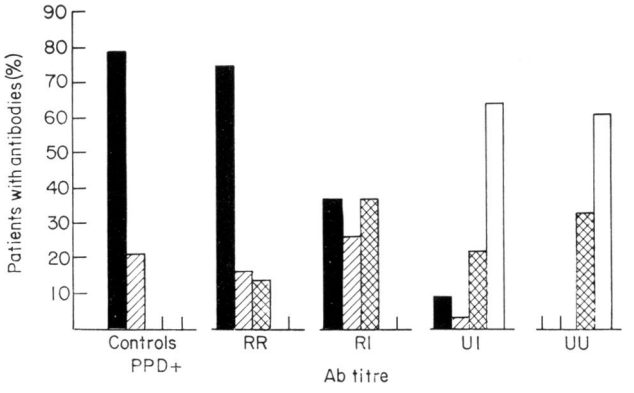


FIG. 2. Haemagglutinating antibody titres in the four clinical groups of patients showing increasing antibody levels with the more unreactive form of disease. Ab titre: solid columns, negative; hatched columns, 1:8, cross-hatched columns, 1:8–1:32; open columns, 1:32–1:128 and over.

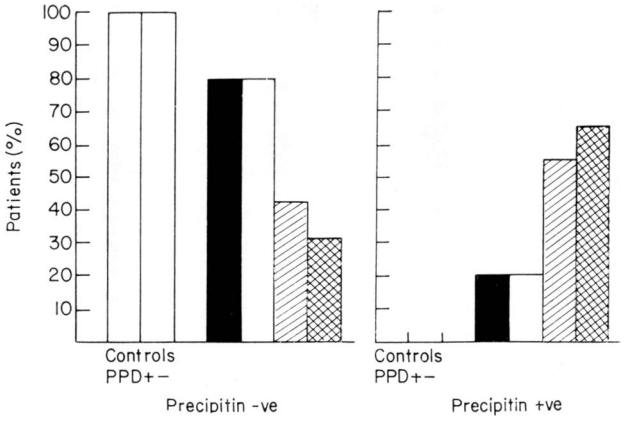


FIG. 3. Precipitin tests in two patient groups. In general only the unreactive (UI and UU) patients had a positive precipitin test. Solid columns, RR; open columns, RI; hatched columns, UI; cross-hatched columns, UU. Healthy controls (PPD +ve and −ve): open columns.

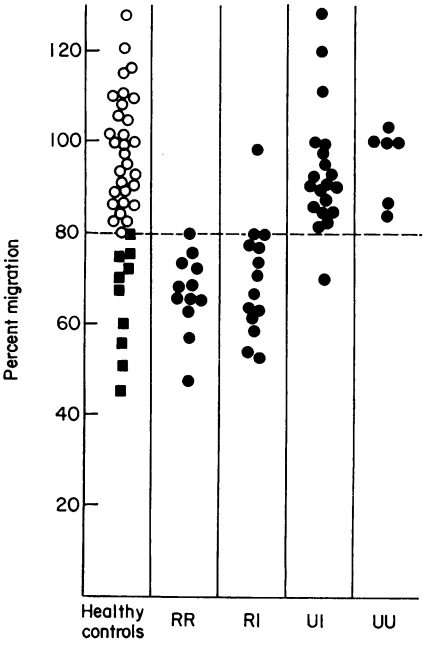


FIG. 4. Leucocyte migration inhibition tests (PPD 125 μg/ml) in healthy PPD positive and negative controls, and the four groups of patients. The migration inhibition is absent in the unreactive groups UI and UU and present in RR and IR. (○) PPD−; (■) PPD+, healthy controls.

Comparison of in vitro and in vivo tests

The comparison between circulating antibody, migration inhibition and *in vivo* skin testing with PPD shows clear distinctions between the groups of patients and reflects the main differences in immune responsiveness to the mycobacterium.

RR, reactive. In this group of patients with localized lesions and a prompt response to chemotherapy, migration inhibition correlates well with the classical delayed skin test and antibody is of low titre or absent. Three typical examples are shown in Fig. 6.

RI, reactive intermediate. Cavitation with surrounding inflammation is a hallmark of this group of

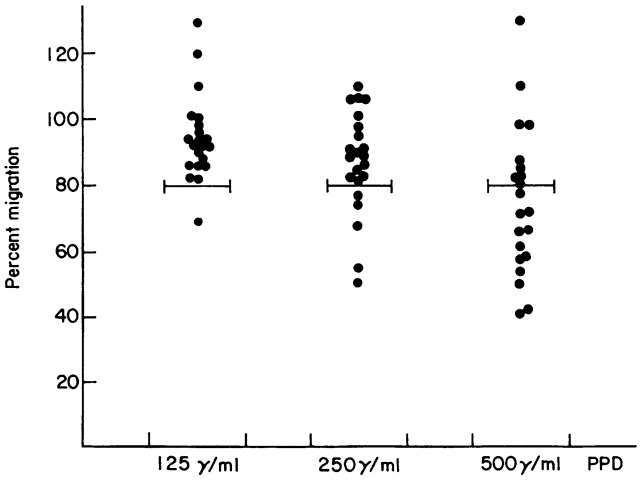


FIG. 5. The dose response of PPD in the migration inhibition test in the unreactive intermediate (UI) group of patients showing increasing inhibition with the increasing dose of antigen. No other groups showed this change in reactivity with antigen dose.

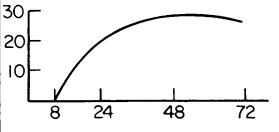
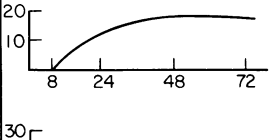
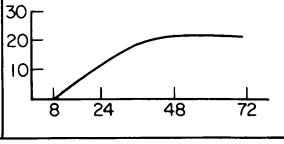
No	Anti-PPD antibodies		LMT to PPD 125 γ/ml	Skin test 5 tu PPD
	Haemagglutination	Immunodiffusion		
5	Negative	-ve	63%	
8	1:4	-ve	65%	
10	Negative	-ve	62%	

FIG. 6. Three representative patients of the Reactive (RR) group showing positive migration inhibition and classic delayed skin tests. Antibody is of low titre or absent.

patients, although only a single cavity may be present. These patients also repond well to chemotherapy. The main immunological change is that of the skin test, where an early phase (Jones–Mote) is seen as well as the late response. Migration inhibition is still positive, and the antibody titre is low rather than absent. Three examples are shown in Fig. 7.

UI, unreactive intermediate. Chronicity is the hallmark of this group of patients who show cavitation with surrounding fibrosis. For the first time, migration inhibition is negative and precipitins are present. The precipitins are reflected in a high haemagglutination titre. A typical delayed skin reaction is only rarely seen, Jones–Mote or mixed reactions being the rule. Representative examples are shown in Fig. 8.

UU, unreactive. Rapid diffusion of the lesions within the chest and to other organs and a very poor response to treatment is seen in this group. Migration inhibition is absent, precipitins are strongly positive and haemagglutinating antibody is present in high titre. The absolute hallmark in this group is

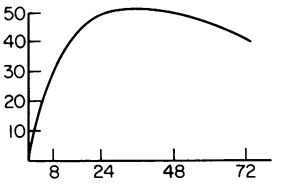
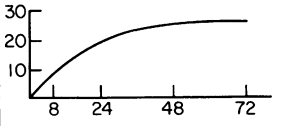
No	Anti-PPD antibodies		LMT to PPD 125 γ/ml	Skin test 5 tu PPD
	Haemagglutination	Immunodiffusion		
2	1:4	Negative	67%	
3	1:32	Negative	54%	
5	1:32	Negative	63%	

FIG. 7. Patients representative of the reactive intermediate (RI) group. Skin tests show mixed Jones–Mote and delayed type reactions. Antibody titres are low rather than absent.

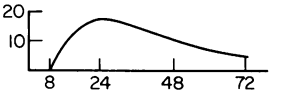
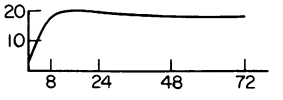
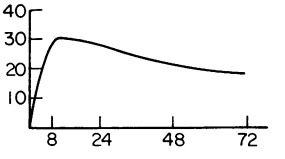
No	Anti-PPD antibodies		LMT to PPD 125 γ/ml	Skin test 5 tu PPD
	Haemagglutination	Immumodiffusion		
3	>1:512	Positive	120%	
9	>1:128	Positive	69%	
11	1:256	Positive	100%	

FIG. 8. Examples of patients in the unreactive intermediate (UI) group where in general migration inhibition is absent, precipitins are present and skin tests show mixed Jones–Mote and delayed reactions.

the absent skin test to even high concentrations of PPD. Three examples are shown in Fig. 9. These cases are rare.

DISCUSSION

Clinical, immunological and morphological data confirm that in human tuberculosis there is a spectrum with two polar forms, reactive and unreactive tuberculosis (RR and UU). The reactive form (RR) is characterized by localized lesions with lymphocytes and epithelioid cells and by a marked early response to antitubercular drugs.

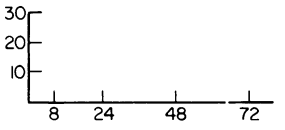
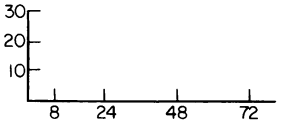
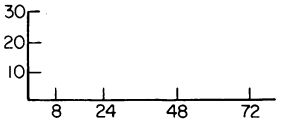
No	Anti-PPD antibodies		LMT to PPD 125 γ/ml	Skin test 100 tu PPD
	Haemagglutination	Immunodiffusion		
1	>512	Positive	85%	
2	1:32	Positive	83%	
3	1:126	Positive	100%	

FIG. 9. Examples of three patients in the unreactive (UU) group where migration inhibition is absent, precipitins are strongly positive and skin tests are negative to even high doses of PPD.

Immunologically, this form shows evidence of active cell-mediated immunity with little or no antibody response. In particular, the skin test to PPD is that of a typical delayed hypersensitivity response and this is reflected in the positive cellular responses *in vitro*.

The unreactive form (UU) is characterized by rapid diffusion of the lesions within the chest and to other organs and a poor response to treatment. Immunologically this polar group shows a very poor or indeed absent cell mediated immune response, in that both skin test and LMT are negative. On the other hand, the antibody response is abundant with a high haemagglutination titre and strong precipitins being present.

The intermediate forms show characteristics of the neighbouring polar groups. The intermediate reactive (IR) group, although showing good cellular responses *in vitro*, does show skin tests somewhat characteristic of Jones–Mote hypersensitivity. The intermediate unreactive (UI) patients show poor cellular reactions *in vitro*, and can show typical Jones–Mote skin tests.

As in leprosy, the intermediate forms are unstable, in that they have the tendency to shift to the contiguous positions after anti-tubercular drug treatment (UI→RI→RR); as a consequence of the secondary immunological depression of host resistance, the shifting is sometimes reversed (RR→ RI→UI).

What is particularly interesting in this context is the reaction of the UI group in the LMT. Here, with a high dose of antigen, previous non-responders do show migration inhibition (Fig. 5). It might be expected that those patients who do respond in this way would show a more prompt response to treatment than the others. This is currently being investigated.

It has long been suspected that there would be a spectrum in tuberculosis similar to leprosy and we feel that this has now been amply demonstrated by us. Previous to this, the classification of tuberculosis has been cumbersome and unnecessarily complicated. Complicated because the classification has been solely based on radiology and histopathology, e.g. Renault, 1971. With immunological features it is now possible to simplify this classification into the four groups described (Table 1). We realise, of course, that these groups represent only arbitrary points on a continuous spectrum of immune responsiveness. With the description of these four relatively homogeneous groups, it will be possible to study this world-wide disease in greater detail and possibly aid in its treatment. Two examples spring to mind. In our experience, a short course of corticosteroids in conjunction with chemotherapy may be helpful in the treatment of RI forms of the disease, because of the reduction in the local non-specific inflammatory response. Secondly, it would be interesting to speculate what effect transfer factor would have in either of the unreactive forms.

TABLE 1. The spectrum of human tuberculosis, as defined on the basis of clinical, bacteriological, histological and immunological data

		Reactive (RR)	Reactive intermediate (RI)	Unreactive intermediate (UI)	Unreactive (UU)
Skin test to PPD	Typical delayed reaction (%)	100	30	5	—
	Early reaction (%)	—	13	15	—
	Mixed reaction (%)	—	57	80	—
Leucocyte migration inhibition		+++	++−	±−−	−−−
Humoral anti PPD antibodies (%)		5	70	98	100
Mycobacteria	In sputum	−−−	−−−	++−	+++
	In the tissue	−−−	+−−	+++	+++
Immunologic change in lymphonode	Germinal centres and plasma cells	−−+	−−+	+++	−−+
	Paracortical area	+++	++−	+−−	−−−
Response to antimycobacterial treatment (%)		100	90	33	0

We would like to thank Dr J. Brostoff for his help in setting up the immunological techniques and for his help in the preparation of the manuscript. We would also like to thank Professor J. Turk for his continued interest and helpful criticism.

This study was supported by a grant from CNR (no. 730137243).

REFERENCES

BROSTOFF, J. (1970) Migration inhibition studies in human diseases. *Proc. roy. Soc. Med.* **63**, 905.

DADDI, G. & PANÁ, L. (1947) *Recettività e resistenza nella tubercolosi polmonare.* (ed. by Vallecchi), Firenze.

GODAL, T. (1974) The role of immune response to myobacterium leprae in host defence and tissue damage in leprosy. *Progr. Immunol.* **II**, 4.

LEFFORD, M.J., MCGREGOR, D.D. & MACKANESS, G.B. (1973) Immune response to mycobacterium tuberculosis in rats. *Infect. Immunol.* **9**, 182.

LENZINI, L. (1974) Immunita all'infezione: problematica ancora attuale. *Giorn. Batt. Virol. Immunol.* **11**, 67.

LENZINI, L. & BARNABÈ, R. (1969) The nature of changes in cellular infiltration for two weeks after tuberculin testing in patients with active tuberculosis. *Int. Arch. Allergy*, **35**, 402.

LENZINI, L. & BARNABÈ, R. (1973) Contributo dell'attuale metodologia immunologica alle conoscenze dei meccanismi dell'Ipersensibilità Ritardata e per la diagnostica della malattia tubercolare. *Ann. Sclavo*, **15**, 6.

LENZINI, L. & FAENZI, G. (1966) Patomorfologia della tubercolosi polmonare cavitaria cronica. *Rassegna Pat. app. Resp.*, XVI, 2.

LENZINI, L., ROTTOLI, L. & ROTTOLI, P. (1974) Inibizione della migrazione leucocitaria nella tubercolosi e in malattie con anergia cutanea. *Ann. Sclavo*, **16**, 29.

LENZINI, L., ROTTOLI, P., ROTTOLI, L. & SESTINI, S. (1974) Gli anticorpi circolanti liberi anti-PPD nella infezione e nella malattia tubercolare. *Boll. Soc. Ital. Biol. Sper.* **50**, 89.

LURIE, M.B. (1964) Resistance to tuberculosis. Harvard University Press, Cambridge, Massachusetts.

MACKANESS, G.B. (1968) The immunology of antituberculous immunity. *Amer. Rev. resp. Dis.* **97**, 337.

NORTH, R.J. (1973) Importance of thymus derived lymphocytes in cell-mediated immunity to infection. *Cell. Immunol.* **7**, 166.

RENAULT, P. (1971) Etude anatomo-pathologique de la tuberculose polmonaire. *Encyclopédie Medico-Chirurgicale*, (ed. by Pouman), volume 3, page 1, Editions Techniques, Paris.

RICH, A.R. (1941) The significance of hypersensitivity in infection. *Physiol. Rev.* **21**, 70.

ROITT, I.M. & DONIACH, D. (1972) *W.H.O. Manual of Immunology Techniques*, Geneva.

TURK, J.L.B. & BRYCESON, A.D.I.M. (1971) Immunological phenomena in leprosy and related diseases. *Advanc. Immunol.* **13**, 209.

W.H.O. Scientific Group Report (1973) Cell mediated immunity and resistance to infection. *Wld. Hlth. Org. Techn. Rep. Ser.* no. 519, Geneva.

W.H.O. Chronicle (1975) Tuberculosis control: progress of the new strategy. *W.H.O. Chronicle*, **29**, 123.