Clin. exp. Immunol. (1986) **64,** 484–494.

The cellular responses of tuberculosis and leprosy patients and of healthy controls in skin tests to 'New Tuberculin' and Leprosin A

J. SWANSON BECK, SUSAN M. MORLEY, J. H. GIBBS, R. C. POTTS, M. I. ILIAS,* T. KARDJITO,* J. M. GRANGE,† J. STANFORD‡ & R. A. BROWN

University of Dundee, UK, Airlangga University, Surabaya, Indonesia, the † Cardiothoracic Institute, London, UK and ‡ Middlesex Hospital Medical School, London, UK*

(Accepted for publication 9 January 1986)

SUMMARY

The density and distribution of T4 and T8 lymphocytes and of monocyte/macrophages at the site of skin tests with mycobacterial antigens was studied in pulmonary tuberculosis and leprosy patients and in healthy controls. Most of the inflammatory cells were located in perivascular and periappendicular foci in the dermis: the percentage of the dermis occupied by focal infiltrate was unrelated to the clinical measurement of the area of induration. There was a less intense diffuse infiltrate in the dermis between the foci, most marked in the papillary dermis and lessening progressively in deeper layers. In patients, diffusely infiltrating lymphocytes were more numerous (mainly due to an excess of T8 cells) in relation to extracts of the pathogen causing their disease than to extracts of the other organism: T8 cells were particularly numerous in reactions to Leprosin A in three of four partly treated leprosy patients who had been classified as tuberculoid at the time of diagnosis. The density of diffusely infiltrating macrophages showed a similar density gradient and selective concentration in response to active disease pathogens. However these cells were less numerous in partly treated leprosy patients than in controls and most frequent in untreated pulmonary tuberculosis patients. Selective migration of monocyte/macrophages and, to a lesser extent T8 cells, appears to be a prominent feature in the reaction of patient with active mycobacterial disease to antigens derived from the causative organisms: this suggests that it might become possible to distinguish direct reactions from cross-reactions in human delayed hypersensitivity reactions by identification of these histological features.

Keywords skin-tests tuberculosis leprosy lymphocyte-subpopulations macrophages

INTRODUCTION

Skin testing has been used extensively to determine the immune reactivity of subjects towards antigens of *Mycobacterium tuberculosis* and related species. In most positive reactors, the dermal swelling has a time-course of evolution corresponding to that of a delayed (cell mediated or Type IV) hypersensitivity reaction. Histological studies of biopsies taken at 48–72 h with conventional methods (Turk, 1980) and with immunocytochemical methods (Poulter *et al.*, 1982; Platt *et al.*, 1983; Gibbs *et al.*, 1984) confirm that the dominant reaction is a cellular infiltrate characteristic of a

Correspondence: Professor J. Swanson Beck, Department of Pathology, Ninewells Hospital and Medical School, P.O. Box 120, Dundee DD1 9SY, UK.

Type IV reaction. The significance of the tuberculin reaction has, however, been the subject of considerable controversy: some workers consider it to be a manifestation of the protective immune reaction, while others regard it as a distinct reaction irrelevant to, or even antagonistic to, the mechanisms for protection against infection by *M. tuberculosis* (Lefford, 1975; Youmans, 1975).

Recently, the subject of delayed hypersensitivity has been rendered much more complex by the knowledge that the cells involved in the tissue reaction, lymphocytes and monocytes, contain subsets with distinctive physiological functions. Reactions manifesting as dermal swelling need not result from a single mechanism and may vary according to the balance between various lymphocyte subsets recruited and activated by the antigen (Rook, 1983; Grange *et al.*, 1984). The type and behaviour of the cells in the infiltrate could depend critically upon the nature of the antigens present in the skin testing reagent and the current immune status of the subject.

Immunodiffusion analysis of the genus *Mycobacterium* (Stanford & Grange, 1974) has revealed four main groups of antigens: those shared by all species (group I), those found amongst slowly growing species (group II), those occurring in rapidly growing species (group III) and those unique to each individual species (group IV). Each species thus contains groups I and IV and either group II or III antigens, although, a minority of species, notably *M. leprae* and *M. vaccae*, contain the former two groups of antigens only (Stanford *et al.*, 1975a).

Numerous attempts have been made to isolate one or more of the species-specific (group IV) antigens for use in skin testing and serodiagnostic tests, but so far without success (Daniel & Janicki, 1978; Grange, 1984). The chemical identity of the individual epitopes has not yet been established. Accordingly, the distinction between specific reactions and cross-reactivity has traditionally been made by performing simultaneous skin tests with reagents prepared from different mycobacterial species and observing differences in the size of the ensuing induration (Magnusson, 1981).

In most investigations, the only feature of the skin test that is recorded is the diameter of the induration at 48 or 72 h: only recently has serious attention been paid to qualitative differences in the dermal reactions as well as to variation in their size (Kardjito & Grange, 1982; Stanford & Lema, 1983). As the clinically observed types of dermal swelling are the gross morphological end-results of the complex cellular interaction in the test site, the external appearance cannot provide more than an indirect and subjective indication of immune reactivity.

In the hope of obtaining a more direct and objective assessment of specificity in skin test reactions in mycobacterial disease we have analysed the response to New Tuberculin and to Leprosin A in tuberculosis patients, leprosy patients and healthy controls in terms of the density of lymphocytes (and their subsets) and of monocyte/macrophages in the lesion, with particular reference to their microanatomical distribution within the dermis.

MATERIALS AND METHODS

The study was made on three groups of subjects (a) nineteen tuberculosis patients (14 men & 5 women; aged 20–67 years) at the time of confirmation of the diagnosis by chest X-ray and ZN staining of acid-fast bacilli in the sputum, before the start of anti-tuberculous drug treatment, (b) seven patients with leprosy under active treatment (4 originally diagnosed as tuberculoid and 3 as lepromatous, 5 men & 2 women aged 25–45 years) and (c) on 15 healthy control subjects (7 men & 8 women; aged 22–60 years) with no radiological abnormality in the chest and from the same socio-economic group and age range as the patients. All participants had given informed consent without coercion. All were citizens of Surabaya or adjacent parts of East Java (Indonesia). Skin tests were performed with antigens prepared from mycobacteria by ultra-sonic disruption and filtration (termed 'New Tuberculins'—Paul *et al.*, 1975; Stanford *et al.*, 1975b; Shield *et al.*, 1977; Anonymous, 1984). The reagents used in this study were prepared from cultures of *M. tuberculosis* (Tuberculin), *M. vaccae* (Vaccin), *M. scrofulaceum* (Scrofulin) and from armadillo-derived *M. leprae* (Leprosin A). All tuberculosis and leprosy patients were tested with Tuberculin and Leprosin A: all the controls were tested with Tuberculin, some were tested with Leprosin A and some with Vaccin or Scrofulin. The diameters of the areas of erythema and induration in the long axis of the forearm and at right angles were measured to the nearest mm with a ruler at 48 h.

Each subject had a biopsy taken with a 4 mm disposable skin punch (Stiefel Laboratories Ltd., Slough, UK) from the centre (corresponding to the injection sites) of each of the two selected reactions under local anaesthesia with 0·5 ml 2% plain lignocaine. Each specimen was divided in two: one half was snap-frozen for immunocytochemistry and the other half was fixed in 4% neutral buffered methanal (formaldehyde) for conventional histology (Gibbs *et al.*, 1984). After return to Dundee, the frozen blocks were cryopreserved with 5% glycerol (Coghill *et al.*, 1985) and 6 μm sections were cut in a cryostat. The sections were fixed briefly in propan-2-one (acetone), rehydrated and treated with commercial monoclonal antibodies: Leu-1 (anti-T1), Leu-3a (anti-T4), Leu-2a (anti-T8), and Leu-M3 (anti-monocyte/ macrophage)(Becton Dickinson, Sunnyvale, CA, USA). Thereafter the sections were treated with Vectastain kit (containing biotinylated antimouse immunoglobulin and the third stage reagent, avidin/biotinylated peroxidase) (Sera-Lab Ltd., Crawley Down, Sussex, UK); finally a histochemical method was used for the generation of the brown reaction product from diamino-benzidene (Gibbs *et al.*, 1984).

The method for quantitative analysis of the extent and localization of the chronic inflammatory infiltrate in the dermis used in this investigation was modified slightly from that described in detail previously (Gibbs *et al.*, 1984). Briefly, each section was photographed and monochrome photographic prints (265 × 210 mm) were prepared showing the whole area of the section at a magnification of ×83. The margins of the focal areas of exudate (where the reticular dermal collagen was stripped off the vessels or appendages) were usually sufficiently clear in the photographs, but in case of difficulty, the location of the margin of the condensed reticular collagen was easily identified by direct observation in a polarising microscope: the edges of the focal infiltrate

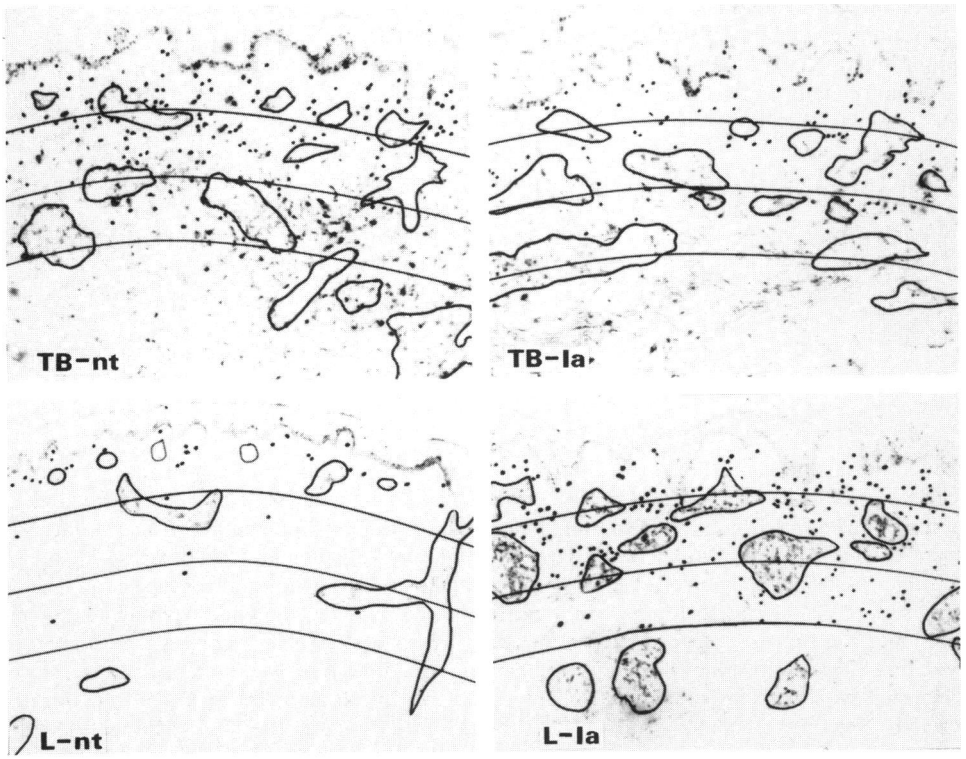

Fig. 1. Comparison of distribution of T8 bearing lymphocytes in the dermis of a tuberculosis patient (upper pair) and a leprosy patient (lower pair) at the site of skin test with New Tuberculin (left) and Leprosin A (right). The positions of individual stained cells in the diffuse infiltrate have been emphasized with dense markers, the edges of the foci of inflammation have been outlined and lines have been drawn to show the positions of the successive 240 μm levels into the dermis. It is clear that the density of T8 cells is greater in the reaction of the patients to extracts from the organism causing their disease.

were then delineated on the photograph with a felt pen (Fig. 1). Area measurements were made by cursor planimetry (Imagan, Graphic Information Systems, Blairgowrie, UK) and corrected to absolute units according to the magnification factor. For each section the following areas were measured: (a) that of the whole of the dermis, (b) those of each focus and (c) that of the intervening dermis (i.e., the area between the foci) in successive 240 μm layers downwards from the dermo-epidermal junction. The number of cells in each of the upper three layers of the intervening dermis was counted and their density expressed as number/mm^2.

Analyses were performed to determine the extent and statistical significance of (a) differences in the density of cells in the diffuse infiltrate at various levels into the dermis and (b) differences between responses to different antigens in the three subject groups: two-way analysis of variance was chosen since this approach allows for correlation between measurements in adjacent levels and could, if necessary, accommodate a logarithmic transformation to stabilize the variance. This type of analysis effectively breaks the total variability in the data into components attributable to (a) differences between levels, (b) differences between subject/antigen responses and (c) variability attributable to neither of these. Standard variance ratio tests were used to assess the significance of any apparent differences. The calculations were performed on a DEC 10 mainline computer using the Generalised Linear Interactive Modelling Package produced by the Royal Statistical Society (Baker & Nelder, 1978).

RESULTS

Focal infiltrate in dermis

The vast majority of the inflammatory cells were located around blood vessels and skin appendages as focal areas of exudate. Measurement of the area of such foci allows the calculation of the volume proportion of the dermis affected by this concentrated inflammatory reaction at the site of biopsy

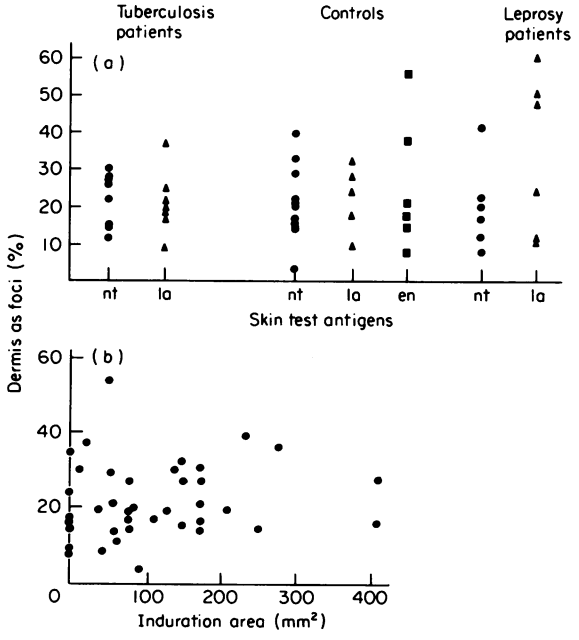

Fig. 2. Histometric measurements of focal infiltrate in skin tests to mycobacterial extracts. (a) Shows that the intensity of this component of the reaction was similar in all groups and was not related to the antigen used for skin testing. (nt) Tuberculin; (la) Leprosin A; (en) Vaccin and Scrofulin. (b) A scatter diagram to demonstrate that there is no obvious relationship between the percentage of the dermis occupied by focal inflammation, judged histologically and the area of induration measured clinically 48 h into the skin reaction to testing with various 'New Tuberculins'.

and this is a histometric index of the intensity of the inflammatory response at the centre of the reaction. Figure 2 compares the extent of focal infiltrate determined histologically with the diameter of the area of induration measured clinically for all the biopsies in this study: it is clear that there is no correlation (Spearman's rank correlation, $r = 0{\cdot}02$). When the reactions to individual antigens in the three subject groups were considered separately no clear pattern was seen since the observations in the subgroups were scattered quite widely with considerable overlap (Fig. 2). The predominant cell type was the T lymphocyte, T4 cells were more numerous than T8 cells: about one third of the cells carried the phenotypic marker of monocyte/macrophages and all three types of cells were apparently randomly intermixed within each focus.

Lymphocyte infiltrate in dermis between foci

Histological examination of the sections indicated that lymphocytes were most numerous in the most superficial dermis and that their density decreased progressively in deeper levels of the dermis (Fig. 1). This pattern of cell distribution was present in both tuberculosis and leprosy patients and the controls in response to both antigens extracted from pathogenic mycobacteria (Tuberculin and Leprosin A) and environmental organisms (Scrofulin and Vaccin). This 'gradient' of decreasing density with increasing depth into the dermis was highly significant ($P < 0{\cdot}0001$) in analysis of variance on the counts of cell density in all the biopsies in this study.

Amongst patients with tuberculosis the overall density of lymphocytes (*i.e.*, the sum of the densities of T4 and T8 cells) was significantly greater in their response to Tuberculin than to Leprosin A (Table 1). Conversely, patients with leprosy showed a greater density of lymphocyte infiltrate to Leprosin A than to Tuberculin (Fig. 1). These differences were due mainly to the increase in numbers of T8 cells in the responses to antigens derived from the organism responsible for the patient's disease (Fig. 3, Table 1): the density of T4 cells was not statistically different in the various reactions. There was a very much lower T4/T8 ratio in the diffusely infiltrating lymphocytes in the reactions of the tuberculosis patients to Tuberculin than to Leprosin *A* ($P < 0{\cdot}001$) and *vice*

Table 1. Results of analysis of variance on the histometric counts of lymphocytes in the diffuse component of the dermal infiltrate

		Mean density in reaction (cells/mm²)				Summary of significant differences in counts*		
Cells	Level	TB-nt	TB-la	L-nt	L-la	Comparison group	Difference	*P* value† less than
All (T4+T8)	1	463·0	301·8	296·5	632·5	TB-nt/TB-la	nt > la	0·001
lymphocytes	2	321·5	210·4	191·5	390·4	TB-nt/L-nt	TB > L	0·005
	3	226·5	149·4	140·8	289·7	TB-la/L-la	L > TB	0·005
						L-la/L-nt	la > nt	0·01
T8 lymphocytes	1	135·8	55·6	71·0	243·1	TB-nt/TB-la	nt > la	0·001
	2	101·2	38·0	29·6	173·9	TB-nt/L-nt	TB > L	0·001
	3	65·3	28·8	35·6	115·4	TB-nt/C-nt	TB > C	0·001
T4/T8 ratio	1	2·61	5·81	3·88	2·76	TB-nt/TB-la	la > nt	0·001
	2	3·21	4·92	5·72	2·95	L-nt/L-la	nt > la	0·001
	3	3·06	6·32	3·42	2·33			

* Comparison of all other patient/antigen groups (including environmental mycobacteria) gave results which were statistically not significant at the 5% level.

† *P* value for analysis of variance.

Subjects: (TB) tuberculin patients; (C) healthy controls. (L) leprosy patients.

Antigens: (nt) new tuberculin; (la) leprosin A.

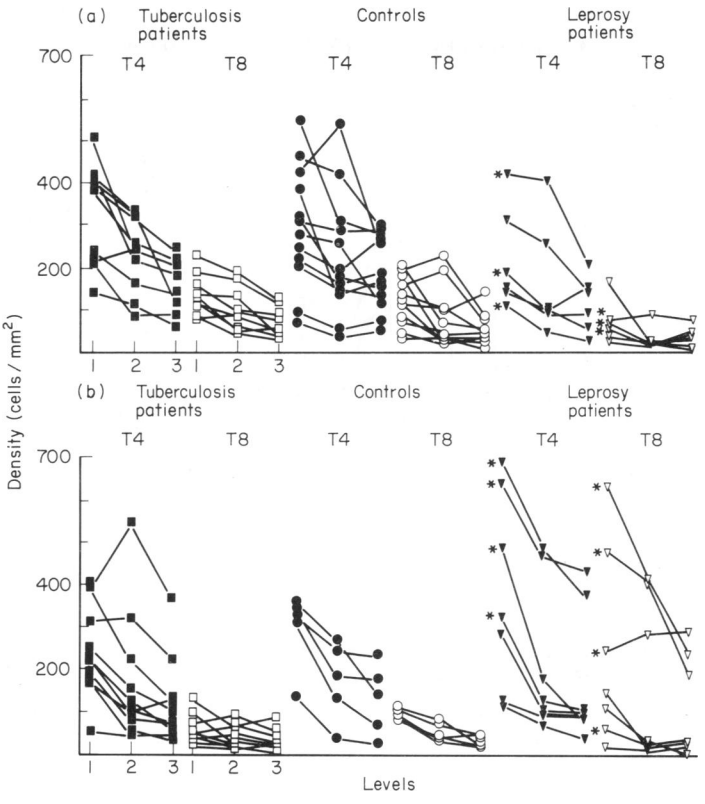

Fig. 3. The density of T4 and T8 lymphocytes in successive 240 μm layers of the dermis 48 h after skin testing with (a) Tuberculin and (b) Leprosin A. (nt) Tuberculin antigen; (la) Leprosin A antigen; (en) environmental mycobacterial antigens (Scrofulin or Vaccin); (*) leprosy patients originally diagnosed as having tuberculoid form of the disease.

versa in the responses of leprosy patients ($P<0{\cdot}001$) (Table 1). The leprosy patients showed considerable scatter of density of diffusely infiltrating lymphocytes in their reactions to Leprosin A: the three patients whose lesions had many more T8 cells had all been diagnosed as having the tuberculoid form of the disease before treatment was started, whereas three of the four patients with the lesser T8 infiltrate had been classified as lepromatous leprosy before treatment was started (Fig. 3).

Monocyte/macrophage infiltrate in the dermis between foci

The monocyte/macrophage cells were most numerous in the superficial dermis and had a density gradient similar to that of the lymphocytes: histometry has confirmed this trend (Fig. 4) which is highly significant in all the groups studied ($P<0.001$). The Tuberculin reactions of the tuberculosis patients had many more diffusely infiltrating monocyte/macrophages than the corresponding reactions in both the controls and the leprosy patients. In addition, the tuberculosis patients had a denser monocyte infiltrate in their reactions to Tuberculin than to Leprosin A. The control subjects did not show any differences in their monocyte densities in reactions to Tuberculin, Leprosin A, Vaccin or Scrofulin.

The leprosy patients had very few diffusely infiltrating macrophages in their reactions to Tuberculin. They had relatively more macrophages in their reactions to Leprosin A, but, even in this reaction, the numbers were no greater than in the various reactions of the control subjects: the responses of the leprosy patients were much less intense than those of the tuberculosis patients. The full results of this statistical analysis are shown in Table 2.

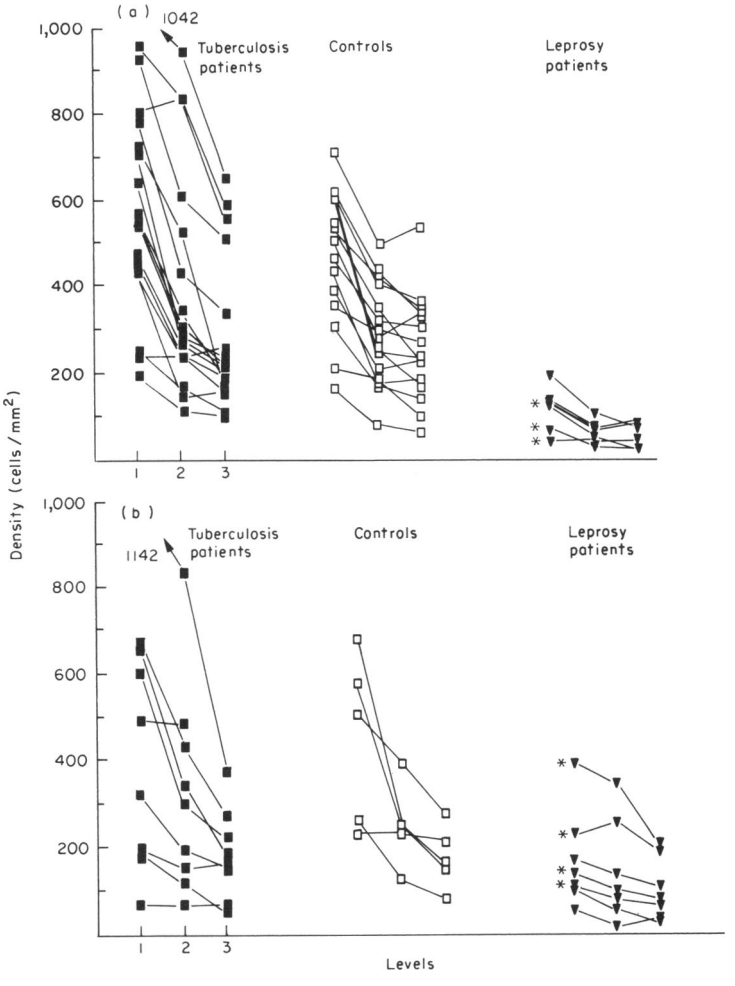

Fig. 4. The density of monocyte/macrophage cells in successive 240 μm layers of the dermis 48 h after skin testing with (a) New Tuberculin antigen and (b) Leprosin antigen.

DISCUSSION

Previous studies have indicated that the density of lymphocytes and monocyte/macrophages in the foci of exudate do not vary markedly from patient to patient during the first 96 h of the reaction (Gibbs *et al.*, 1984). Since the vast majority of the inflammatory cells in skin test responses to mycobacterial antigens are located in the foci (> 90%, unpublished observations), it was concluded that the proportion of the dermis occupied by these foci would be an indication of the number of cells that had emigrated from the blood into the tissues and so could be used as a histometric index of the intensity of the tuberculin reaction at the biopsy site (the site of antigen injection in this study). Clinically, the diameter of the area of induration is generally regarded as indicating the severity of the reaction. It was clear from Fig. 2 that these two measurements showed no correlation. Indeed, there were some reactions that had quite extensive focal cellular infiltrates but no induration and these would be regarded as negative on clinical examination. Similar discrepancies between the extent of focal cellular infiltrate and the degree of induration have been observed in the responses of certain Indian leprosy patients to Leprosin A (Samuel, Stanford & Desikan, 1985) and in a London study of clinically negative tuberculin reactions in sarcoidosis patients (Mishra *et al.*, 1983).

Table 2. Results of analysis of variance on the histometric counts of monocyte/macrophages in the diffuse component of the dermal infiltrate

Reaction		Mean density of monocyte/macrophages (cells/mm²) at levels			Summary of significant differences in counts*		
Subject group	Antigen	1	2	3	Comparison group	Difference	*P* value† less than
TB	nt	583·8	388·1	275·9	TB-nt/TB-la	nt > la	0·01
TB	la	484·4	326·1	223·0	TB-nt/L-nt	TB > L	0·0001
C	nt	446·3	286·3	247·8	TB-la/L-la	TB > L	0·01
C	la	453·6	266·0	237·8	L-nt/L-la	la > nt	0·0025
C	en	517·0	326·2	268·2			
L	nt	109·3	58·0	51·0			
L	la	175·4	160·6	106·3			

* Comparison of all other patient/antigen groups gave results that were statistically significant at the 1% level or better, except for TB-nt/C-en, TB-la/C-nt, TB-la/C-la, TB-la/C-en, C-nt/C-la, C-nt/C-en and C-la/C-en.

† *P* value.

Subjects: (TB) tuberculosis patients; (C) healthy controls; (L) leprosy patients.

Antigens: (nt) new tuberculin; (la) leprosin A; (en) environmental antigen.

Moreover, it is possible to inhibit the clinical manifestations of the classical Mantoux reaction by direct suggestion under hypnosis without affecting the cellular infiltration (Black, Humphrey & Niven, 1963). These various studies indicate that the cellular infiltrate and the induration are distinct phenomona. The latter result from vascular changes consequent on release of a variety of vasoactive mediators. It has been shown that a minority of individuals exposed to mycobacteria fail to develop a dermal response to the corresponding antigens (Stanford *et al.*, 1981): the group of non-responders differed from the responders in that none of them possessed the HLA-DR3 histocompatibility antigen (van Eden *et al.*, 1983). This raises the possibility that there may be a genetically determined modulation of release of mediators after an adequate recruitment of cells.

It was noteworthy that there were more diffusely infiltrating lymphocytes (mainly T8s) and monocyte/macrophages in the reactions of patients to extracts of the organisms responsible for their disease than to other mycobacteria: tuberculosis and leprosy patients showed a reciprocal relationship in this respect. While it is appreciated that the Indonesian patients with leprosy commonly have contact with open tuberculosis patients and to a lesser extent *vice versa*, none of the patients had both diseases: histometric evidence from the patients therefore suggests that there is a specific component to the cellular migration, possibly invoked by activation of clones of cells responding to the species-specific (group IV) antigens of the mycobacterium responsible for the disease. Clearly, this could be biologically advantageous in concentrating defensive cells around invading pathogens. On the other hand, it could lead to specific recruitment of suppressor cells or cells involved in tissue-destroying hypersensitivity reactions to the detriment of the host.

Biopsies of the leprosy lesions in the patients who participated in this study had an 'indeterminate' appearance at the time of skin testing, probably because they had been treated with dapsone and other drugs for 3–27 months. It was, however, noteworthy that three of the four patients originally diagnosed as tuberculoid leprosy showed many more migrating T8s whereas the single remaining tuberculoid and all three lepromatous patients did not show an excess of this phenotypic subset of lymphocytes. Selective migration of lymphocyte subsets under immune stimulation does not appear to have been demonstrated previously and this may be an important determinant in the selective localisation of various cell types in the lesion of the different types of leprosy (Modlin *et al.*, 1983). It is tempting to postulate that the T8 cells exert a selective 'damping down' effect in order to prevent excessive tissue damage. Care must, however, be taken in relating the expression of phenotypic surface antigen to cell function. There is evidence that there are subsets of both T4 and T8 cells with quite different functions, although these cannot yet be identified. Thus, T4 cells activated by BCG have been shown to suppress T cell proliferation induced by various mycobacterial antigens (Mustafa & Godal, 1983). Furthermore, there is a suggestion that T8 cells from patients with lepromatous leprosy are defective in their ability to suppress mitogen-driven lymphocyte responses (Rook, 1982). There is increasing evidence that the range of functions of T cells is not confined to those usually ascribed to their phenotypes. Thus a single clone of T cells was found to mediate B cell help, cytotoxicity and delayed hypersensitivity reactions (Dennert, Weiss & Warner, 1981). Although cloned T cells may not behave in the same manner as those *in vivo*, T cells of the Lyt-1^+, 2^- phenotype (corresponding to T4 cells in man) mediated both helper and suppressor functions in mice infected with *Leishmania tropica* (Liew, Hale & Howard, 1982): dose titration studies suggested that there were two functional subpopulations within this single phenotype.

Finally, it was remarkable that there were relatively few monocyte/macrophages in the leprosy patient's skin tests compared with those of the tuberculosis patients and controls. This contrasts with the very large numbers of these cells in the natural lesions of leprosy: it suggests that they accumulate more slowly and this could contribute to the slow evolution of the disease and its chronic nature.

In conclusion, the study of the cellular nature of biopsies of the skin test site in patients with mycobacterial diseases yields information of a quite different nature to that provided by a clinical measurement of the area of dermal induration. Such techniques will, hopefully, prove to be powerful tools for the study of the complex cellular interactions in such diseases and for the assessment of the cellular reactivity of the patients.

This work was supported by a generous grant from the Wellcome Trust. We are grateful to Dr D.S. Ridley for review of the sections of the skin lesions of the treated leprosy patients at the time of skin testing and to Dr R.J.W. Rees who supplied the armadillo-derived *M. leprae* for the preparation of Leprosin A. Mr R.S. Fawkes prepared the diagrams and Mrs Rosalind Mitchell gave us valuable secretarial assistance.

REFERENCES

ANONYMOUS (1984) New tuberculins. *Lancet*, **i**, 199.

BAKER, R.J. & NELDER, J.A. (1978) Generalised linear interactive modelling: release 3. *Royal Statistical Society*, London, UK.

BLACK, S., HUMPHREY, J.H. & NIVEN, J.S.F. (1983) Inhibition of Mantoux reaction by direct suggestion under hypnosis. *Br. Med. J.* **i**, 1649.

COGHILL, G., GIBBS, J.H., LOWE, J.G. & BECK, J.S. (1985) Cryopreservation with glycerol during cryostat sectioning for localisation of lymphocytes and assessory cell phenotypic subsets in tissue biopsies. *J. clin. Pathol.* **38**, 840.

DANIEL, T.M. & JANICKI, B.W. (1978) Mycobacterial antigens: a review of their isolation, chemistry and immunological properties. *Microbiol. Rev.* **42**, 84.

DENNERT, G., WEISS, S. & WARNER, J.F. (1981) T cells may express multiple activities: specific allohelp, cytolysis and delayed hypersensitivity are expressed by a cloned T-cell line. *Proc. natn. Acad. Sci.* **78**, 4540.

VAN EDEN, W., DE VRIES, R.R.P., STANFORD, J.L. & ROOK, G.A.W. (1983) HLA-DR3 associated genetic control of response to multiple skin tests with new tuberculins. *Clin. exp. Immunol.* **52**, 287.

GIBBS, J.H., FERGUSON, J., BROWN, R.A., KENICER, K.J.A., POTTS, R.C., COGHILL, G. & BECK, J.S. (1984) Histometric study of the localisation of lymphocyte subsets and accessory cells in human Mantoux reactions. *J. clin. Pathol.* **37**, 1227.

GRANGE, J.M. (1984) The humoral immune response in tuberculosis: its nature, biological role and diagnostic usefulness. *Adv. Tub. Res.* **21**, 1.

GRANGE, J.M., MITCHELL, D.N., KEMP, M. & KARDJITO, T. (1984) Serum angiotensin-converting enzyme and delayed hypersensitivity in pulmonary tuberculosis. *Tubercle*, **64**, 117.

KARDJITO, T. & GRANGE, J.M. (1982) Diagnosis of active tuberculosis by immunological methods. 2. Qualitative differences in the dermal response to tuberculin in patients with active pulmonary disease and healthy tuberculin-positive individuals. *Tubercle*, **63**, 275.

LEFFORD, M.J. (1975) Delayed hypersensitivity in immunity in tuberculosis. *Am. Rev. Respir. Dis.* **111**, 243.

LIEW, F.Y., HALE, C. & HOWARD, J.G. (1982) Immunological regulation of experimental cutaneous leishmaniasis. V. Characteristics of effector and suppressor cells. *J. Immunol.* **128**, 1917.

MAGNUSSON, M. (1981) Mycobacterial sensitins: where are we now? *Rev. Infect. Dis.* **3**, 944.

MISHRA, B.B., POULTER, L.W., JANOSSY, G. & JAMES, G. (1983) The distribution of lymphoid and macrophage-like cell subsets in sarcoid and Kveim granulomata: possible mechanism of negative PPD reaction in sarcoidosis. *Clin. exp. Immunol.* **54**, 705.

MODLIN, R.L., HOFMAN, F.M., TAYLOR, C.R. & REA, T.H. (1983) T-lymphocyte subsets in the skin lesions of patients with leprosy. *J. Am. Acad. Dermatol.* **8**, 182.

MUSTAFA, A.S. & GODAL, T. (1983) *In vitro* induction of human suppressor T cells by mycobacterial antigens. BCG activated OKT4$^+$ cells mediate suppression of antigen induced T-cell proliferation. *Clin. exp. Immunol.* **52**, 29.

PAUL, R.C., STANFORD, J.L., MISLJENOVIC, O. & LEFFERING, J. (1975) Multiple skin-testing of Kenyan school children with a series of new tuberculins. *J. Hyg. (Lond.)*. **75**, 303.

PLATT, J.L., GRANT, B.W., EDDY, A.A. & MITCHELL, A.F. (1983) Immune cell populations in cutaneous delayed-type hypersensitivity. *J. exp. Med.* **158**, 1227.

POULTER, L.W., SEYMOUR, G.J., DUKE, O., JANOSSY, G. & PANAYI, G. (1982) Immunohistological analysis of delayed-type hypersensitivity in man. *Cell. Immunol.* **74**, 358.

ROOK, G.A.W. (1982) Suppressor cells of mouse and man. What is the evidence that they contribute to the aetiology of the mycobacterioses? *Lepr. Rev.* **53**, 306.

ROOK, G.A.W. (1983) The immunology of leprosy. *Tubercle*, **64**, 297.

SAMUEL, N.M., STANFORD, J.L. & DESIKAN, K.V. (1985) Microscopic findings of delayed reactions elicited by the skin test reagent Leprosin A derived from *M. leprae*. *Int. J. Lepr.* **53**, 395.

SCHNEYNIUS, A., KLARESKOG, L. & FORSUM, U. (1982) *In situ* identification of T lymphocyte subsets and HLA-DR expressing cells in the human skin tuberculin reactions. *Clin. exp. Immunol.* **49**, 325.

SHIELD, M.J., STANFORD, J.L., PAUL, R.C. & CARSWELL, J.W. (1977) Multiple skin testing of tuberculosis patients with a range of new tuberculins and a comparison with leprosy and *Mycobacterium ulcerans* infections. *J. Hyg. (Lond.)*. **78**, 331.

STANFORD, J.L. & GRANGE, J.M. (1974) The meaning and structure of species as applied to mycobacteria. *Tubercle*, **55**, 143.

STANFORD, J.L. & LEMA, E. (1983) The use of a sonicate preparation of *Mycobacterium tuberculosis* (new tuberculin) in the assessment of BCG vaccination. *Tubercle*, **64**, 275.

STANFORD, J.L., ROOK, G.A.W., CONVIT, J., GODAL, T., KRONVALL, G., REES, R.J.W. & WALSH, G.P. (1975a) Preliminary taxonomic studies on the leprosy bacillus. *Br. J. exp. Pathol.* **56**, 579.

STANFORD, J.L., NYE, P.M., ROOK, G.A.W., SAMUEL, N. & FAIRBANK, A. (1981) A preliminary investigation of the responsiveness or otherwise of patients and staff of a leprosy hospital to groups of shared or species-specific antigens of mycobacteria. *Lepr. Rev.* **52**, 321.

STANFORD, J.L., REVILL, W.D.L., GUNTHORPE, W.J.

& GRANGE, J.M. (1975b) The production and preliminary investigation of Burulin, a new skin test reagent for *Mycobacterium ulcerans* infection. *J. Hyg. (Lond.)*. **74,** 7.

TURK, J.L. (1980) Delayed hypersensitivity. In *Research monographs in immunology. 3rd ed.* Vol. 1. Elsevier, Amsterdam, The Netherlands.

YOUMANS, G.P. (1975) Relation between delayed hypersensitivity and immunity in tuberculosis. *Am. Rev. Respir. Dis.* **111,** 109.