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Lysozyme as a measure of cellular dynamics in the lesions of leprosy

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Summary. The levels and distribution of lysozyme-positive cells and exudate were studied in leprosy lesions through the spectrum, in untreated and treated patients, in relapse and in reactions. Altogether 124 skin biopsies were examined by the immunoperoxidase technique. Monocytes, neutrophil-polymorphs and mast cells were the most conspicuous cells seen. Lysozyme proved to be a useful means of indexing renewal of these cells in the lesions. Peak numbers of monocytes were seen in lesions of active lepromatous leprosy (LL) and of tuberculoid leprosy (TT), at poles of opposite immunological performance. In TT the stimulus for recruitment was delayed hypersensitivity (DH). A decline in DH from TT towards the middle of the spectrum, mid-borderline, was accompanied by a fall in monocyte level. Furthermore, reacting lesions due to enhanced DH also had increased numbers of monocytes. On the other hand reactions associated with immunological deterioration were similar to active lepromatous leprosy (LL) and monocyte influx was raised in response to the stimulus of free multiplication of bacilli in both cases. In TT delayed hypersensitivity acted also to promote the rapid transformation of monocytes to epithelioid and giant cells all of which were strongly positive for lysozyme. This was in contrast to much lower levels in histologically similar macrophage-epithelioid cells of BT granulomas. Lysozyme synthesis was not seen in macrophages after ingestion of *M. leprae*. Early foamy change was made conspicuous by lysozyme deposited in phagocytic vacuoles, but old foam cells in regressing lepromas were negative. Lysozyme bound to dead extracellular *M. leprae* but not to viable or intracellular organisms. Dead bacilli or immune complexes appeared to be the stimulus for neutrophil-polymorph recruitment, mainly in reactions.

Keywords: lysozyme, leprosy, macrophages

Of the many secretory products of macrophages it is recognized that lysozyme is the bulk product (Nathan *et al.* 1980). Lysozyme is produced also by neutrophil-polymorphs and other myeloid cells, and by mast cells, and is found in a variety of situations in normal and pathological human tissues (Mason & Taylor 1975). It is secreted continuously by macrophages but production ceases when protein synthesis is inhibited (Gordon *et al.* 1974). The mechanisms that control its synthesis, secretion and intracellular transport are unknown (Gordon 1980), and the function of lysozyme in mycobacterial disease remains in doubt. Lysozyme provides a useful marker for macrophages in inflammation but not in neoplasia (Kindblom *et al.* 1982), and release of

lysozyme has been used as a measure of the activating effects of mycobacteria on macrophages (Lyberg et al. 1982).

Lysozyme in leprosy lesions has been the subject of several small scale studies (Rea & Taylor 1977; Ridley *et al.* 1982; Ridley & Russell 1982; Matsuo *et al.* 1982). Cells comprising tuberculoid granulomas were found to stain with a uniform density while those of lepromatous granulomas appeared only to emphasize phagocytic vacuoles. Rea & Taylor (1977) considered that in tuberculoid leprosy alone serum levels of lysozyme reflected amounts produced by the granuloma.

Recently, Werb and her colleagues have shown that the secretory performance of macrophages whether resident, inflammatory stimulated, or immunologically activated, varies both in type and amount according to the committed differentiated function of the cell at the site of inflammation (Werb 1983). We were interested therefore, in studying lysozyme synthesis and deposition in certain granulomatous diseases with a view to elucidating mechanisms concerned with granuloma formation, maintenance and resolution. Leprosy is the model chosen for this study. The low cytopathic properties of *M. leprae* combined with its capacity to induce in some people a strong immune response provide varying host interactions of activity and regression as well as of immunity. The general tendency of patients with leprosy is to downgrade, with a decline in hypersensitivity, towards a state of anergy. The course may be rapid or protracted and it may be interrupted by shifts in immunity, termed reactions. During this time, the macrophage may undergo profound functional changes.

In this paper we consider the secretion of lysozyme in the granuloma in relation to classification (Ridley 1974), to cell type (Ridley 1981), to activity of the infection (Ridley 1980), and to the main types of reaction (Ridley 1977).

We report that lysozyme can be used to index monocyte cell recruitment to the lesion. This was raised as a result of immunological competence or from increased bacterial multiplication. Lysozyme distinguished functionally competent from functionally incompetent epithelioid cells, and recently recruited macrophages from those which had ingested bacilli.

Material and methods

Patients. Fifty-six untreated patients were included in the study. They were classified as tuberculoid (TT) (eight), borderline tuberculoid (BT) (eight), mid-borderline (BB) (eight), borderline lepromatous (BL) (15), active lepromatous (LL/A) (eight), histoid leproma (LL/H) (five), and hyperactive leproma with exacerbation reaction (LL/ER) (four).

In addition there were biopsies from patients treated with dapsone: eight TT and eight BT after 3 months chemotherapy, five BL after 2 years chemotherapy, 10 LL treated for periods between 2 and 5 years and now in regression (LL/R). There were three patients in relapse (LL/RL) from dapsone resistance. Twenty biopsies of the reactional episodes of LL, erythema nodosum leprosum (ENL) lesions from a former study were included for comparison.

Patients with upgrading or reversal reactions due to enhanced hypersensitivity were graded as six BT, of whom five upgraded to TT and one was static at BT following the reactional episode; and six BL all of whom upgraded to BT. Downgrading reactions which are accompanied by deterioration of the patient, BT to BL were studied in two cases.

Histology. Haematoxylin–eosin stained sections and sections stained for acid-fast bacilli were available from all the biopsies used in this study. These were used for classification, enumeration and examination of bacilli. (Ridley 1974, 1977)

Immunoperoxidase. The PAP method used was of established procedures. Briefly the primary antibody against lysozyme was

made to combine with antigen in the tissue section. A bridge antiserum acted to facilitate the reaction of the second antibody against PAP complex with the primary antibody. The final reactional product was made visible by DAB (Ridley 1983). The method is routine for studies on leprosy and other granulomas which serve as additional controls for the procedure. Biopsies were fixed in a formal, mercuric chloride, acetic acid mixture (10:2:3 parts in 100 ml distilled water) for 1.5 to 3 h and then transferred to 70% alcohol before processing. Control sections included normal rabbit serum in place of the primary antibody and prior absorption of the antiserum with *Micrococcus lysodeikticus* (Difco). In each case control sections were negative.

Analysis. A 40-mm objective and ×8 eyepiece were used in estimating the percentage of positively stained cells. Each field comprised about 500 cells. The whole area of granuloma was assessed (3–4 fields). At this magnification it was possible to note the precise morphological appearance of positive cells and extracellular lysozyme deposited in intercellular spaces of the granuloma. Extracellular lysozyme was estimated separately, + + + marked excess, + + moderate and + weak. Intracellular lysozyme was taken as a measure of synthesis and extracellular lysozyme was taken to indicate secretion in the granuloma. The mean of positively stained cells was obtained for each patient and the mean for each group of patients is that recorded in the tables. Total positive macrophages were noted separately from monocytes and neutrophil-polymorphs.

Additional studies. Cells belonging to the macrophage series were delineated morphologically and by positive acid phosphatase and non-specific esterase. The majority, including epithelioid cells, were positive with OKMI monoclonal antibody indicating the macrophage epitope. Morphology was confirmed by ultrastructure. Details of this part of the study carried out on cryostat sections are to be described in full elsewhere.

Results

Macrophages, neutrophil-polymorphs and mast cells were strongly positive for lysozyme. Monocytes were distinguished by their small size, reniform nucleus, low nuclear–cytoplasmic ratio and irregular shape with pseudopods, indicating high motility. These features were marked after staining for lysozyme when increased density of staining corresponded to increased synthesis. These cells were also strongly positive for acid phosphatase and non-specific esterase.

Cells of dendritic appearance with surface characteristics of Langerhans cells stained weakly with a fine granular pattern. They were found among the lymphocytes of TT but not in LL granulomas, and they were increased in BT following chemotherapy. Epidermal Langerhans' cells were negative (these results are to be reported separately).

Comparative results for lysozyme positive cells in lesions of untreated and treated patients through the leprosy spectrum and in reactions are given in Table 1 and Figs 1 and 2. It appeared that monocytes were increased in TT and LL and neutrophil-polymorphs were seen only in active LL and in reactions. Leprosy bacilli were negative except when extracellular. Extracellular lysozyme was marked only in TT and in reactions where it was associated with cellular necrosis.

Tuberculoid Leprosy (TT)

Increased numbers of monocytes infiltrated the lesions particularly in the subepidermal zone where they appeared among loose clusters of poorly defined epithelioid cells and Langhan's giant cells all of which stained strongly with a granular and diffuse appearance (Fig. 3). The demarcation of compact epithelioid cell tubercles with a peripheral lymphocytic zone was usually seen in the deep dermis. The epithelioid cells of the

Table 1. Lysozyme distribution in the granuloma of untreated patients, relapse and reactions (summary)

Leprosy	EC/GC	MØ without AFB	MØ + AFB	Foam	Monocytes	PMNS	Con. tissue MØ
TT	+++	+++	−	−	+++	−	−
BT	++	++	−	−	+	−	−
BB	−	±	−	−	±	−	−
BL	−	−	+	+	−	−	−
LL/A	−	−	++	+	+++	++	−
LL/H	−	−	+		++++	+++	−
LL/R	−	−	−	−	−	−	−
LL/RL	−	+	−	+	+	++	+++
Reactions							
BT→TT	+++ (necrosis)	−	−	−	++++	+++	+
BT–BT	+	+	−	−	++++	++	+
BL→BT	−	+++ (necrosis)	−	−	++++	++++	(necrosis)
BT→BL	−	+	−	−	+	+++	++++

Lysozyme-positive macrophages are acid phosphatase and non-specific esterase positive. Abbreviations: TT = tuberculoid; BT = borderline tuberculoid; BB = mid-borderline; BL = borderline lepromatous; LL/A = active lepromatous; LLH = histoid hyperactive leproma; LL/R = regressing leproma; LL/RL = relapse leproma; BT→TT and BL→BT = upgrading reactions due to immunological enhancement; BT→BL = downgrading reaction due to deterioration of immunity; BT–BT = Static outcome of reaction; EC/GC = Epithelioid cell, giant cell; foam = foam cell (macrophage).

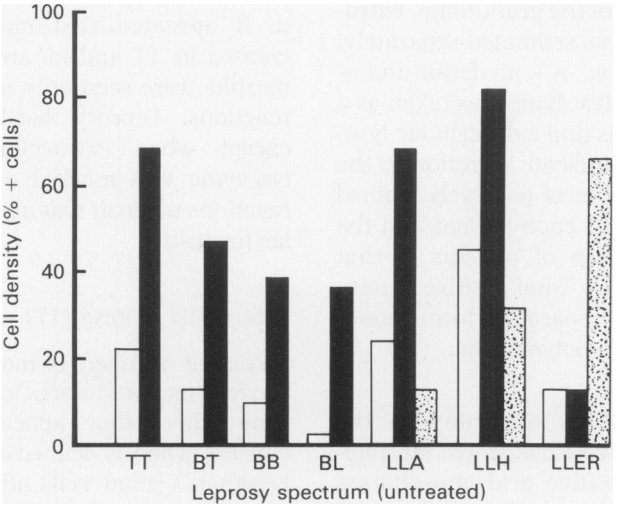

Fig. 1. Levels of cells positive for lysozyme in untreated patients. Total lysozyme-positive cells of the MPS (■), of monocytes (□) and neutrophil polymorphs (▨) reveal high levels of synthesis by activated macrophages and EC's in TT, and binding to phagocytic vacuoles in LL/A. Monocyte influx is raised in TT and LL/A. Polymorphs appear only at the lepromatous pole. Extracellular lysozyme (secretion) is marked only in TT. (not shown on graph).

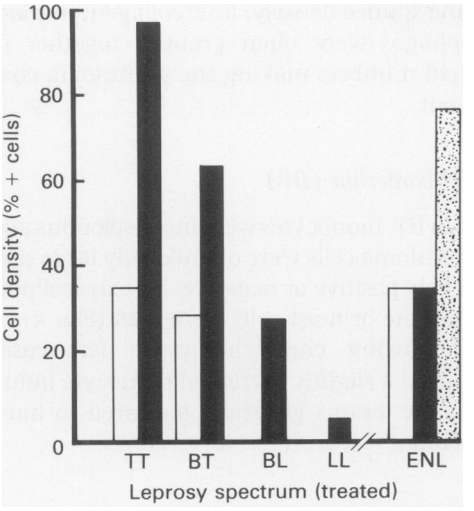

Fig. 2. Levels of cells positive for lysozyme in treated patients and in regressing lesions. Total levels in MPS (■), in monocytes (□), and neutrophil polymorphs (▩) reveal increased monocyte influx in BT compared to untreated patients. MPS cells in BT and TT are smaller in size and strongly positive. Regressing foam cells are negative (LL).

compact tubercle were mixed, mature and immature, the immature cells being strongly positive for lysozyme and the mature cells generally weak or negative. Monocytes accumulated around capillaries at the periphery of compact tubercles but in the upper dermis they were diffusely infiltrative and capillaries were both increased and spaced at random. Intercollagenous macrophages were prominent and recognized by a characteristic staining of lysozyme-positive granules close to the nucleus. Monocytes were not usually seen among them. Cells of similar appearance were seen spread about the margin of compact tubercles. Mast cells were abundant. Extracellular lysozyme was marked in the interstices and on fibrils of the basal lamina.

Following chemotherapy five of eight cases had increased numbers of densely stained monocytes, small activated macrophages, epithelioid cells and giant cells arranged loosely. Necrosis of individual cells

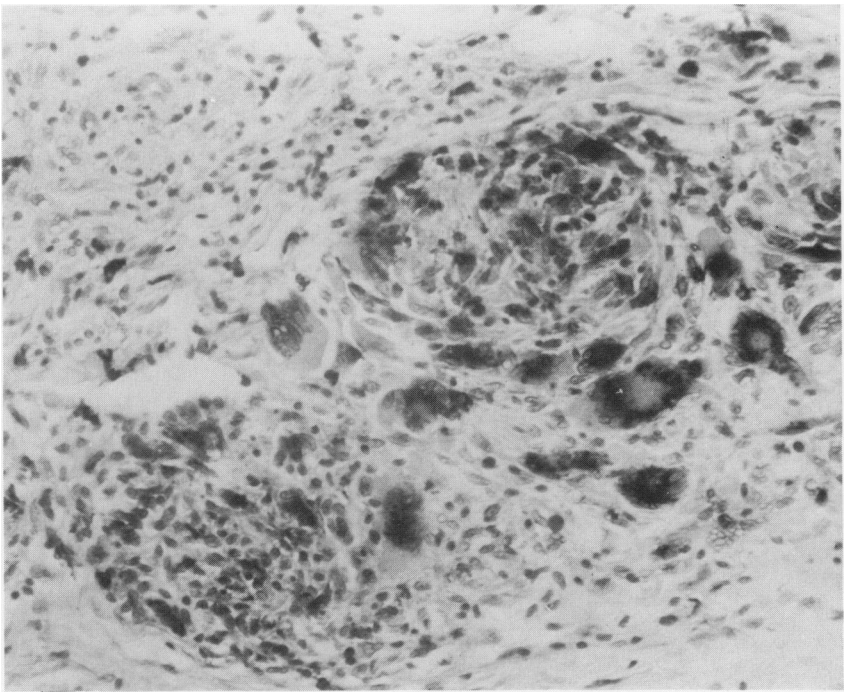

Fig. 3. TT. Granuloma cells are all strongly positive. Monocytes are increased. Extracellular lysozyme is present. × 500.

was common, sometimes focalized. Widely distributed mast cells were seen but intercollagenous macrophages were notably reduced. The remaining three cases show five monocytes per section with small numbers of strongly positive epithelioid cells in compact tubercles.

Borderline tuberculoid (BT)

The granuloma was formed of a sheet of cells with a suggestion of organization into tubercles. The influx of monocytes was reduced (Fig. 4). Granuloma cell size was variable and small amounts of lysozyme-positive granules were seen in the cytoplasm of some cells, others being negative. There was no extracellular lysozyme. Capillaries were spread about the periphery and were often inconspicuous. A few mast cells were present.

Following chemotherapy there was a striking increase in monocyte recruitment at the same time as small activated macrophages, and epithelioid cells of the granuloma stained densely. Intercollagenous macrophages were often grouped together in small numbers making the granuloma confluent.

Mid-borderline (BB)

As in BT, monocytes were inconspicuous and granuloma cells were of uniformly large size, weakly positive or negative. No extracellular lysozyme or mast cells were seen (Fig. 5).

Following chemotherapy a few cases showed a slightly increased monocyte influx but the lesions generally appeared to have shrunk.

Borderline lepromatous (BL)

Monocytes were not seen. Macrophages containing bacilli were negative and formed the main cell type of the upper dermal lesion. In the deep dermis small numbers of foam cells sometimes occurred and more commonly there were cells with a 'foamy' appearance

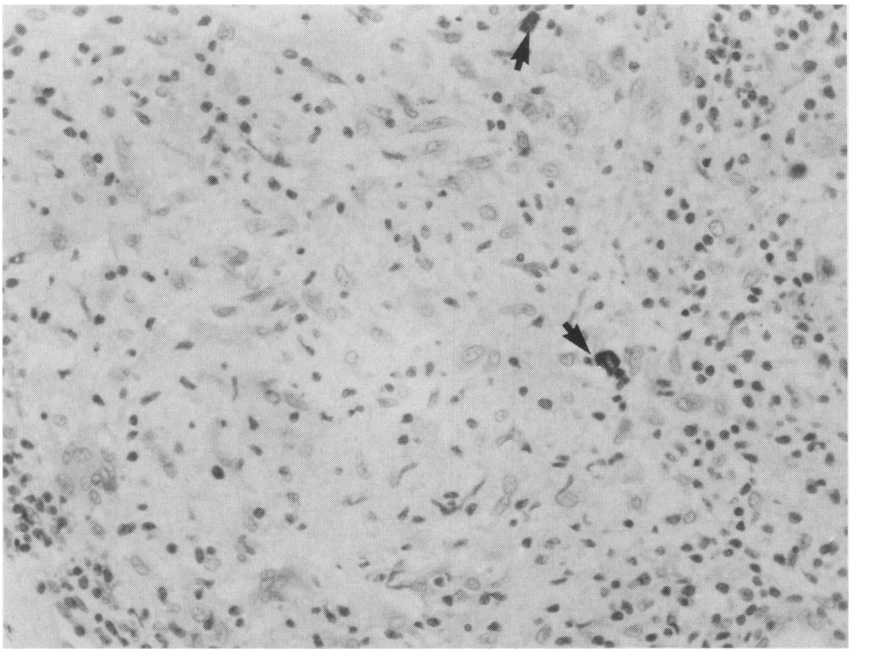

Fig. 4. BT. Granuloma composed of a range of macrophage-epithelioid cells that are weakly positive. No monocytes are seen. Two mast cells are strongly positive (arrows). × 500.

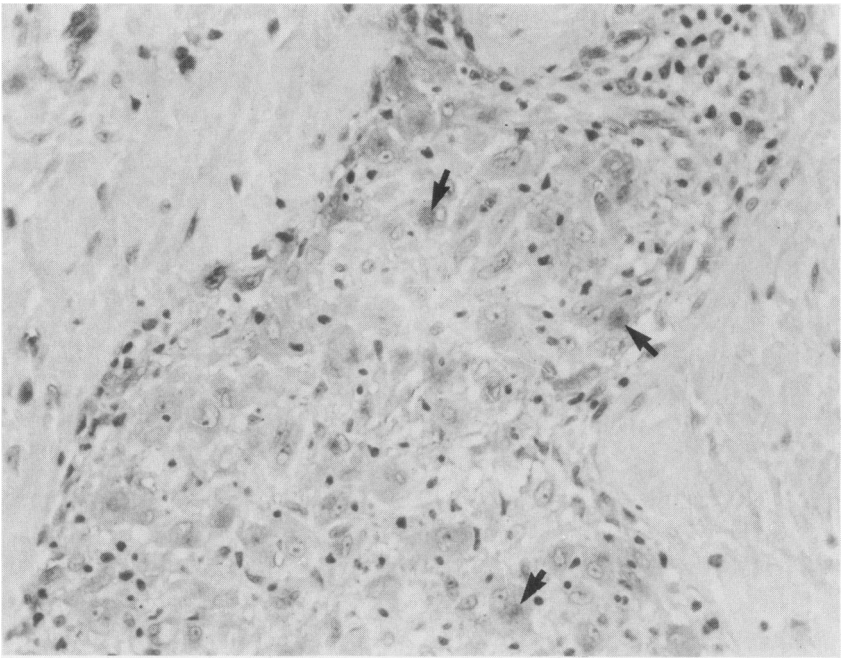

Fig. 5. BB. Granuloma composed of uniformly large cells of MPS with fine intracellular lysozyme granules (arrows). No monocytes are seen. × 500.

due to the binding of lysozyme to phagocytic vacuoles, which was not visible by H and E staining. In some areas there were masses of cells without bacilli which stained weakly. Extracellular lysozyme was absent. Mast cells were sometimes very numerous.

After 2 years chemotherapy the main residual features were foam cells among lymphocytes, but no monocytes.

Lepromatous (LL)

In active lesions (LL/A) strikingly large numbers of monocytes were diffusely scattered among granuloma cells and accumulated in and around small blood vessels (Fig. 6). Some cells infiltrated the subepidermal zone up to the basal layer of the epidermis. Intact polymorphs were seen among them. Large macrophages containing bacilli were negative. These were the commonest granuloma cells. Foam cells were present in the deep dermis and the majority had deposited lysozyme bound to phagocytic vacuoles. Monocytes were rarely seen among them. Individual cells were necrotic but extracellular lysozyme was not detected. Mast cells were common.

After 2 to 5 years treatment the lesions were in regression (LL/R). No monocytes were seen and foam cells were weak or mostly negative (Fig. 7).

Histoid (LL/H)

More than 40% of the lesional cells were monocytes. They were seen among cells containing bacilli which had varying amounts of lysozyme or were negative. Sometimes accumulations of monocytes could be seen around blood vessels which were conspicuous, either peripheral or central in an expansile granuloma. In close association with monocytes were large numbers of polymorphs some of which formed micronecrotic foci. Early 'foamy' change was rare. Mast cells were variable and extracellular lysozyme was sometimes

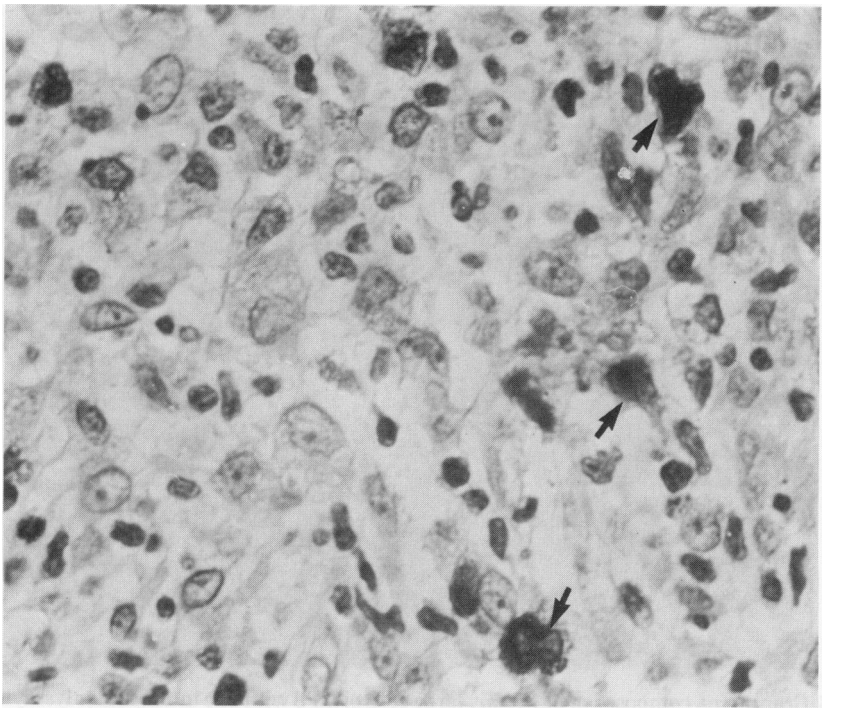

Fig. 6. LL/A. Macrophages are negative with strongly positive monocytes among them (arrows). × 700.

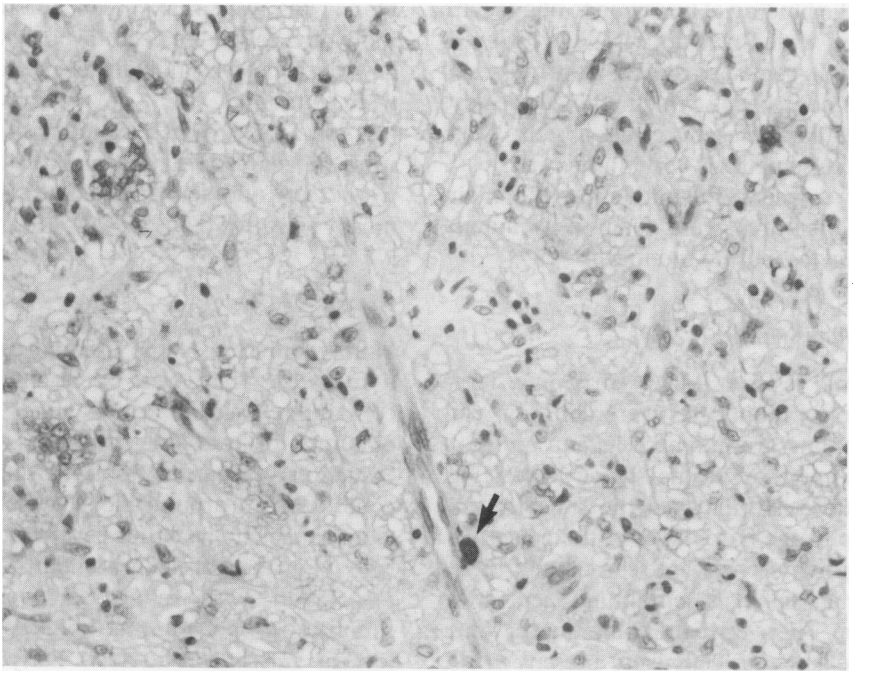

Fig. 7. LL/R. Activity declines. Monocytes are not seen. Old foam cells become progressively negative. One mast cell is strongly positive (arrow). × 250.

marked in the basal lamina and around blood vessels.

Relapse (LL/RL)

The highest activity was seen around the edge of granuloma masses where increased numbers of large strongly positive macrophages with a staining pattern of intercollagenous macrophages were found. Between the collagen there were small accumulations of these cells but monocytes were not found among them. Monocytes were seen in small numbers around blood vessels or within the granuloma especially in the upper dermis. Foam cells and other macrophages containing bacilli were pale or negative. Polymorphs were easily detected in some lesions.

LL/ER (Reaction associated with active infection)

In exacerbation reactions monocytes were notably fewer than in LL/A or LL/H and polymorphs were the commonest lysozyme-positive cells seen. Macrophages containing bacilli were cytoplasmically healthy looking cells that were weakly positive or negative for lysozyme and foam cells were difficult to distinguish. Early 'foamy' change was apparent. The very numerous mast cells were degranulated and aggregated in groups around blood vessels. Extracellular lysozyme was present and heavy in areas of polymorph necrosis and karyorrhexis, as well as in the basal lamina where polymorphs and monocytes accumulated along the basement membrane.

ENL (Immune complex-type reaction)

Large numbers of monocytes were seen among the more common polymorphs in the reacting area. They also appeared dispersed in the exudate fluid. Extracellular lysozyme was abundant in the area of necrosis and far removed from the reaction site, in intercollagenous spaces. Resolution revealed residual positive foam cells and macrophages with ingested lysozyme-positive debris.

Upgrading reactions (associated with enhanced immunity)

BT→TT. The height of the reaction was marked by necrosis involving whorls of epithelioid cell–macrophage tubercles in a confluent granuloma with monocytes and polymorphs predominating at the periphery or at the centre respectively. Extracellular lysozyme and cells of the reacting tubercles were strongly positive, but elsewhere the granuloma cells were weak or negative. Mast cells were very numerous and widely distributed. Lysozyme-positive macrophages and small giant cells were found in groups in the intercollagenous spaces of connective tissue.

BL→BT. At the height of the reaction there were increased numbers of monocytes in connective tissue, which was one of the main sites of reaction (Fig. 8). The edge of the granuloma was very active, with strongly positive activated macrophages, monocytes and polymorphs; some large positive cells had a vesiculated appearance. Elsewhere foam cell masses were infiltrated only by lymphocytes. Necrosis was sometimes extensive, and a constant feature involving the granuloma or more particularly, connective tissue, with a marked increase in extracellular lysozyme and polymorphs. At the end of the reaction infiltrating monocytes were very numerous but granuloma cells were variable, weak or negative for lysozyme. Fibrosis was usually present.

Static reaction

There was no necrosis but a grossly raised level of infiltrating monocytes in the granuloma and in the intercollagenous spaces. Granuloma cells were uniformly pale for lysozyme.

Downgrading reactions (associated with loss of immunity)

BT→BL. In contrast to upgrading reactions, monocytes were not obvious but there was a

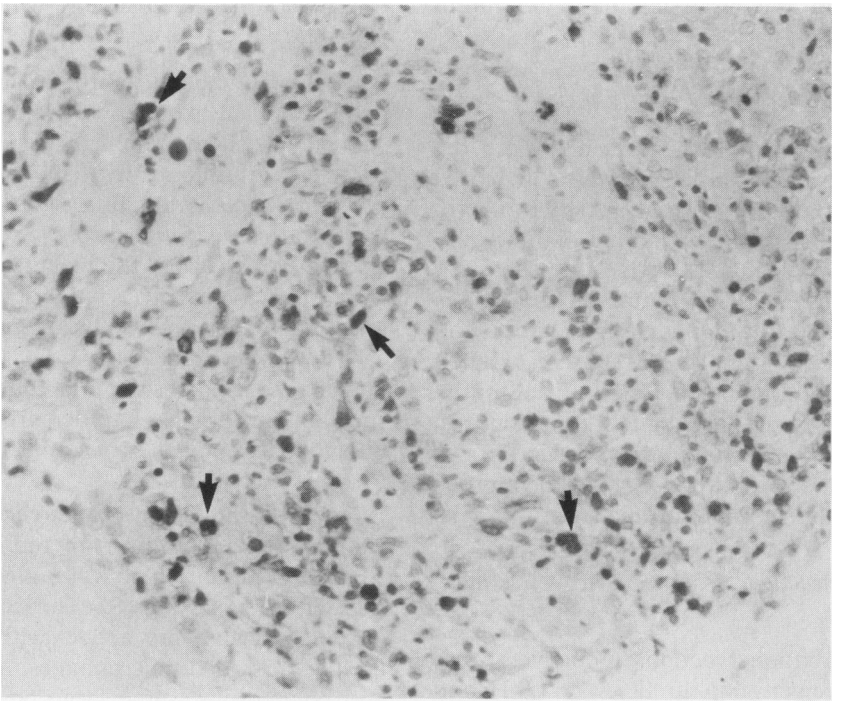

Fig. 8. BL-BT. Monocytes are greatly increased especially in connective tissues (arrows). × 250.

uniform increase of large macrophages at the periphery of the granuloma and epithelioid cells were weak or negative (Fig. 9). These macrophages appeared to be proliferating in small groups in intercollagenous spaces. They stained with a pattern characteristic of these cells, with lysozyme granules close to the nucleus. Polymorphs were sometimes present and mast cells were scattered about the connective tissue.

Discussion

Conspicuous intracytoplasmic staining for lysozyme in leprosy lesions occurred in cells of the mononuclear phagocyte series (MPS), in polymorph-neutrophils and in mast cells. It indicates synthesis of lysozyme by these cells, while deposition of extracellular lysozyme in the interstices of the granuloma or connective tissue fibrils is a mark of secretion or cellular disintegration at these sites.

Concerning the cells of the MPS, the most densely stained were the monocytes. In this series two peaks of lysozyme-positive cells were observed at opposite poles of the spectrum: in the most active lepromatous (LL) lesions lysozyme was seen in large amounts in freshly recruited monocytes; in tuberculoid (TT) lesions it was present both in monocytes and in the rapidly maturing epithelioid and giant cells of the granuloma. It appeared to us that there was one feature in common between these immunologically opposed situations, and that was the high rate of cellular recruitment and proliferation. We conclude that lysozyme serves as a marker and index of the rate of cell renewal in the granuloma.

According to Ryan & Spector (1969), toxicity, dose and distribution of the irritant within the granuloma cells are the key factors in the characterization of the granuloma as high or low cell turnover type; and the ultimate fate of the lesion depends on the persistence of the irritant and the lifespan of

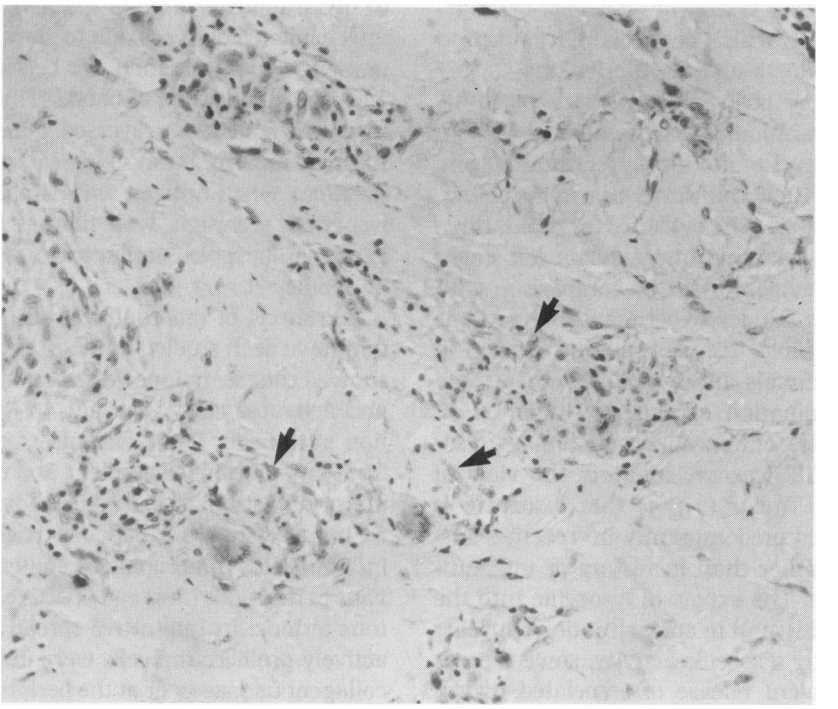

Fig. 9. BT-BL. Monocytes are not conspicuous. Intercollagenous macrophages are increased (arrows). Epithelioid cells and giant cells are negative. × 250.

the MPS cells. In the light of our findings with lysozyme it would appear that in leprosy the perpetuating stimulus responsible for the high cell turnover is either delayed hypersensitivity to *M. leprae* (since the organism itself is almost totally lacking in cytopathogenicity), or it is the uncontrolled multiplication of these bacilli under low immune conditions. If there is a common factor in the initiation of these developments it may be in the role of colony stimulating factor, to which immature macrophages respond, (Nicola & Vadas 1984).

In lepromatous (LL) leprosy the lysozyme level was related to the activity of the infection: lysozyme was present mainly in monocytes, and the rapid influx of these biosynthetically active but immunologically uncommitted, undifferentiated cells facilitates the continued multiplication of *M. leprae*. In due course the ratio of dead to live organisms rises progressively and it may be this that terminates the phase of hyperactivity, with a fall in the rate of recruitment. Mackaness *et al.* (1973) noted that viable, not dead, BCG potentiated the immune response. Apart from monocytes, lysozyme was deposited in the phagocytic vacuoles of macrophages. This made visible the early phase of foamy change; but old foamy cells, which constitute the majority of cells in regressing lepromatous granulomas, were lysozyme-negative. On ingestion of *M. leprae* protein synthesis in the macrophage is progressively reduced along with Fc and C_3 receptor expression (Birdi *et al.* 1979; Ridley *et al.* 1978) and lysozyme synthesis ceases, thus confirming the earlier report (Gordon *et al.* 1974). The continuous release of lysozyme *in vitro*, by all cells of the MPS irrespective of external stimuli (Gordon *et al.* 1974), was not confirmed in our study *in vivo*. It may depend on the establishment of high or low cell turnover states, on the integrity of the

microvascular system or on a loose arrangement of cells which permits the local action of cell products such as interleukins

The other peak level of lysozyme, in TT leprosy granulomas, was associated with an elevated level of monocyte recruitment and with their rapid transformation to the epithelioid cell stage. The evidence for rapid transformation was that intermediate cell stages were less evident in TT by comparison with BT lesions. Under experimental conditions such a rapid transformation occurs in primed animals and is linked with a more rapid elimination of antigen (Ridley *et al.* 1983). The enhanced production of lysozyme in TT leprosy, supports the view of Mason & Taylor (1975) that lysozyme is synthesized predominantly in 'reactive' histiocytes rather than in resting or unstimulated cells. The export of lysozyme into the blood and lymph in such situations appears to be partly a specific activity, since there is no equivalent release of associated hydrolases (Carr *et al*, 1980).

Concerning epithelioid cells, increased lysozyme production as found here in TT leprosy may indicate stimulation rather than activation in cells that are not phagocytic and probably not engaged in cidal activity. Lysozyme is known to have an antibacterial property and is likely to be secreted along with hydrogen peroxide and plasminogen activator which are produced by activated cells for cidal effect, but cells which are activated in this way, may not be exactly those stimulated for lysozyme secretion; it may be that a particular regulation is maintained among stimulated and activated cells for the elimination of antigen and for homeostasis. Sonicated components of *M. leprae* have been shown to stimulate macrophages to release large amounts of lysozyme (Lyberg *et al.* 1982) and such soluble substances may be present in TT lesions. But epithelioid cells of BT and BB leprosy, although morphologically identical with the cells of TT, had a demonstrably lower level of lysozyme secretion. This was associated with the persistence of viable *M. leprae* segregated in macrophages and a greater range of intermediate cell types due to slow transformation. Conditions then are better than in TT for multiplication of bacilli. This low cell turnover state was reversed after chemotherapy and in immunological upgrading reactions when antigen was eliminated and lysozyme secretion was increased in the extracellular space, similar to the situation in TT (Ridley *et al.* 1982).

Secretions of macrophages in inflammation have been studied by Werb (1983) who showed that secretions varied for stimulated and activated cells according to their function at the site. There was also some difference in secretions of resident and of inflammatory macrophages. In our study it looked as if the distinction between resident and inflammatory macrophages might be relevant to the mode of spread of active lepromatous lesions. In infiltrative spread the most actively proliferating cells were in the intercollagenous spaces, or at the periphery of the main granuloma, and they stained with the pattern of resident macrophages. (Further confirmation of this point is being sought in enzyme studies particularly 5'nucleotidase.) There was a similar proliferation of these cells in downgrading reactions due to deteriorating immunity, but the characteristic cell of upgrading reactions due to enhanced immunity and increased DH was the inflammatory monocyte. Thus lysozyme staining may be useful in identifying these reactional states.

A recent review concerning the two opposite phenomena, macrophage inhibition factor (MIF) and macrophage migration stimulation factor (MStF), produced respectively by helper T lymphocytes and suppressor/cytotoxic lymphocytes, is of interest in view of our observations on macrophages *in situ* in leprosy lesions (Fox *et al.* 1981).

In the immune complex ENL reaction of lepromatous leprosy, lysozyme was present in polymorphs, and release of lysozyme resulted in high local levels. There was no impairment of recruitment, as suggested by Sher *et al.* (1978). The stimulus for their

recruitment appeared to be dead rather than live organisms. This suggested that lysozyme might be playing a role in the lysis of dead extracellular leprosy bacilli and their peptidoglycan cell walls. Lysozyme was seen to bind to dead extracellular bacilli but it appeared to be quickly neutralized and lost if these bacilli became re-ingested by a macrophage. In this it differed from C-reactive protein which binds strongly to *M. leprae* and its products (Ridley *et al.* 1984).

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