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Studies of Cell Death (Apoptosis) and Cell Division in Leprosy Granulomas[1]

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The histology of skin lesions in leprosy is well documented and has been found to correlate with both clinical and immunological features of the disease. Histological classification of the skin lesions is based on many factors, including the cellular composition of the granulomas, the number of bacilli present, and the appearance of the mononuclear phagocytes ([9]). A biopsy from the edge of an established lesion is most likely to contain the largest and most characteristic granulomas and, therefore, biopsies from this area are used for diagnostic purposes ([9]). Pathological changes in areas other than at the edge of the lesion have received relatively little attention. However, central resolution of leprosy skin lesions is apparent both clinically and histologically ([1, 9]), and abnormalities of the apparently unremarkable skin away from the edge of the lesion have also been reported ([3, 8, 10, 11]).

The pathological processes underlying these striking differences within the same lesion are not known, but it seems likely that they reflect a balance between cell gain and cell loss in different parts of the lesion. If cell loss predominates, then the lesion will regress, but when cell gain outstrips cell loss the lesion will increase in size. Increased cell gain may also be seen as increased histological activity ([9]). The balance of this cell turnover will, in turn, reflect other factors such as bacillary load, release of antigen and degree of cell-mediated immunity. Unlike many other granulomatous diseases, resolution of leprosy lesions is not usually accompanied by appreciable fibrosis ([9]). Resolution of experimental granulomas in animals is associated with loss of epithelioid cells ([7]), but the mechanism behind this phenomenon has not been elucidated. One possible mechanism of cell loss is apoptosis, a recently recognized mode of cell death resulting in the loss of cells from living tissues ([4, 5, 13]). In apoptosis, the individual cells condense and become transformed into membrane-bound eosinophilic bodies without provoking any inflammatory reaction ([4]); by contrast, necrotic cells undergo lysis and provoke local inflammatory reaction ([13]).

Cell gain within experimental granulomas is accomplished by cell migration into the lesion and mitosis of cells already there. In order to investigate cell turnover within leprosy lesions, we have studied the histological changes present at the center, edge, and outside of leprosy lesions using histometric techniques.

MATERIALS AND METHODS

Patients. Biopsies were taken from 25 patients (16 male, 9 female) attending the Dr. Bandorawalla Hospital, Kondhwa Budruk, Pune, Maharashtra, India. The mean age of the patients was 32 years (S.D. = 13.7 years). Informed consent was obtained from the patients, all of whom were untreated or had received treatment (WHO triple therapy) for less than 1 month.

Biopsies. Each biopsy was taken aseptically using a 4 mm disposable skin punch (Stiefel Laboratories [U.K.] Ltd., Slough, England) under local anesthesia with 1% plain lignocaine. In each case, biopsies were obtained from the center of the lesion, the edge of the lesion, and from apparently uninvolved skin outside the lesion at a point 2 cm from the nearest identifiable margin. The biopsies were fixed in 4% neutral buffered formaldehyde and transported to Dundee, Scotland, for analysis. Each biopsy was bisected; one half was embedded in paraffin wax, the other in glycol methacrylate ([12]).

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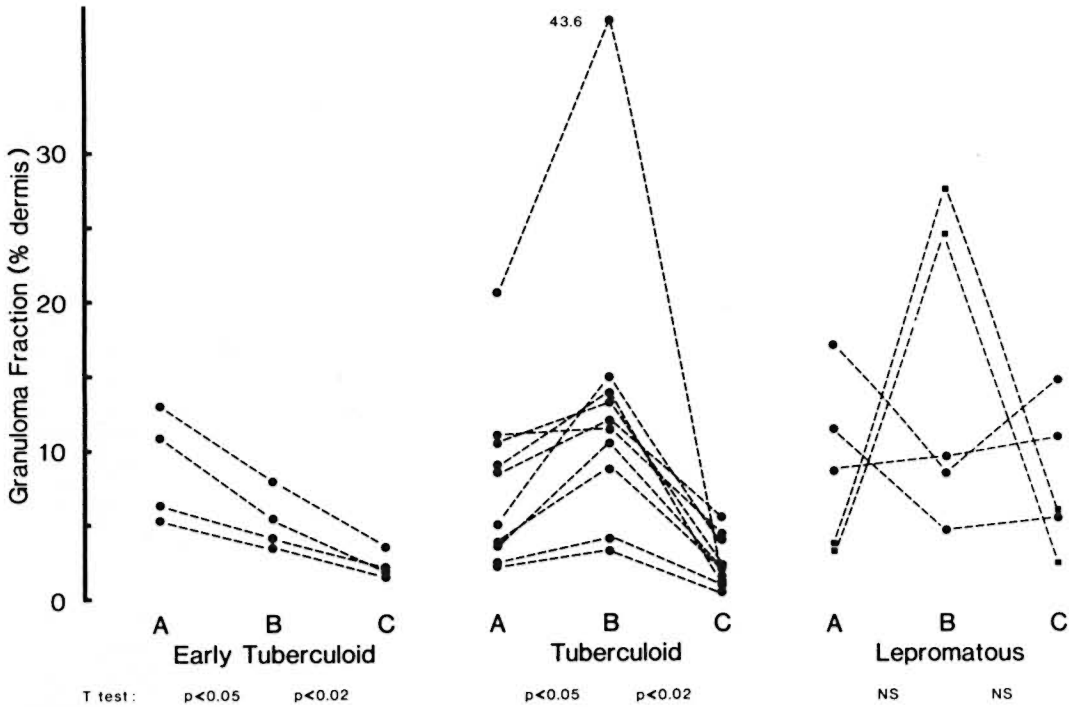

FIG. 1. Granuloma fraction measurements for biopsies from the center (A) and edge (B) of the lesion and from clinically unremarkable skin 2 cm from the lesion (C). ■ = Results from cases classified as BL.

The paraffin sections (5 μm) were stained with hematoxylin and eosin (H&E) and by the Wade-Fite method for diagnosis. Biopsies taken from the edge of the lesion were classified on the Ridley-Jopling scale as: BT = 14, BL = 2, and LLs = 3. Six cases showed indeterminate histology and were excluded. The remaining patient showed evidence of the local exacerbation reaction and was excluded because of the possibility of confusion of neutrophils with apoptotic bodies in the methacrylate sections used for histometry.

Histometry. Methacrylate sections (2 μm) stained with H&E were used for histometric studies. The granuloma fraction (GF) was measured as previously described ([2]). Apoptotic bodies and mitoses were counted in the inflammatory infiltrate at a magnification of ×400 using a 25-square eyepiece grid. Mitoses and apoptoses were identified morphologically, and the number in each section counted. Objects with the general appearance of apoptotic bodies were examined further under oil immersion with the ×100 objective and were accepted as such if they showed two or more of the following morphological features ([4, 14]): a) round/oval structure of variable size separated from surrounding cells; b) acidophilic cytoplasm; and c) one or more fragments of condensed chromatin.

Analysis of results. The number of mitoses or apoptoses is expressed per mm^2 area of granuloma in order to standardize the results and allow comparison of rates in different sections across the lesion and between patients. A statistical analysis of the GF was performed using paired or two-sample Student's *t* tests as appropriate. Apoptosis and mitosis counts were analyzed using a fourfold chi-squared test based on their presence or absence in the material examined.

RESULTS

The results obtained from measurement of the GF (Fig. 1) show that biopsies from the edge of established tuberculoid lesions contained more extensive granuloma than those taken from the center. In the four cases shown as early BT leprosy, the histology at the lesion edge was indeterminate, while the appearances were compatible with a BT

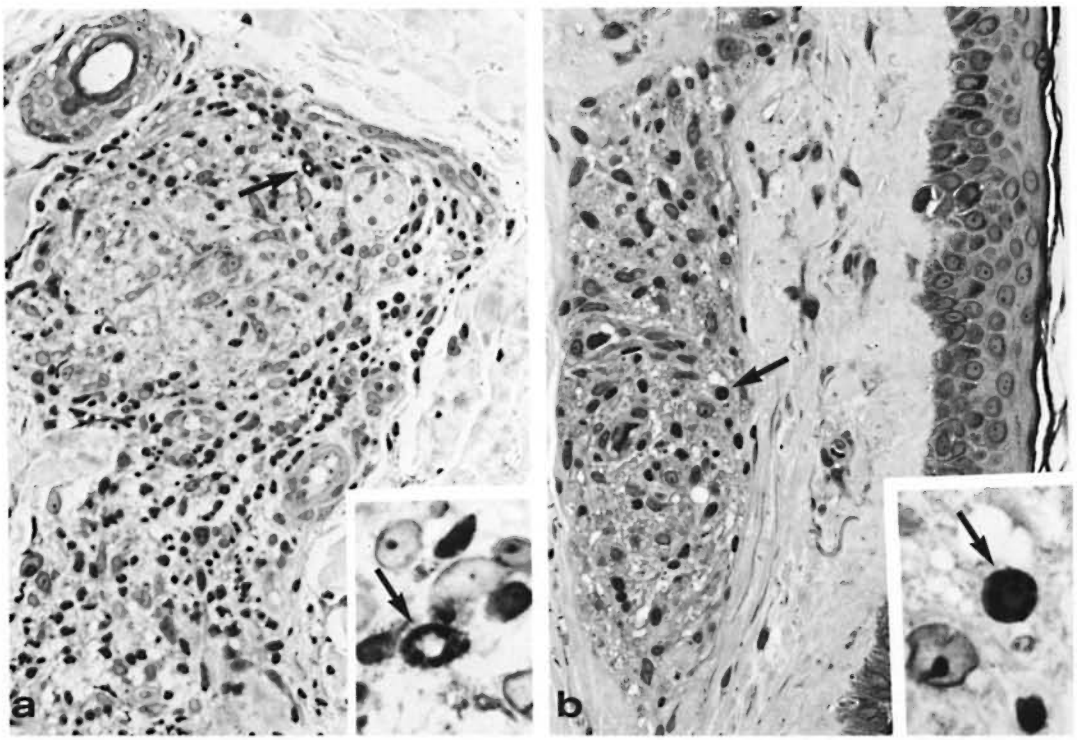

FIG. 2. **a)** Tuberculoid granuloma section (×400) containing an apoptotic body (➡) which is shown at higher power (×1600) in the inset. **b)** Lepromatous granuloma section (×400) containing an apoptotic body (➡) which is shown at higher power (×1600) in the inset.

classification in the central biopsy. In these early BT lesions, the center of the lesion had a higher GF than the edge. By contrast, there is relatively little difference between the center and edge in most lepromatous lesions, although the two BL cases had a higher GF at the edge of the lesion. Outside the lesion, histological changes suggestive of leprosy were seen in the clinically uninvolved skin of 10 of the 14 tuberculoid patients and in all 5 of the lepromatous cases. The GF for biopsies from the clinically unremarkable skin was significantly greater in the lepromatous patients and, histologically, 4 of these 5 showed macrophage granulomas with acid-fast bacilli. The remaining case was classified as BL and showed a mononuclear cell infiltrate around dermal appendages; no bacilli were seen in this case. Similar mild chronic inflammation was apparent in biopsies from outside the lesion in 2 of the early tuberculoid and in 7 of the established tuberculoid cases, 2 of which also contained bacilli.

Unequivocal apoptotic bodies were seen in both tuberculoid and lepromatous granulomas (Fig. 2). These bodies were generally seen in areas composed predominantly of epithelioid cells in tuberculoid cases and in the predominantly histiocytic granulomas in lepromatous cases. In established tuberculoid lesions, the apoptotic count per mm^2 was greater at the edge than at the center of the lesion in all but one case (Fig. 3). In contrast, 3 of the 4 early tuberculoid cases had a high density of apoptotic bodies at the center of their lesions and showed no apoptosis at the edge. In lepromatous cases, the apoptotic count per mm^2 of granuloma was similar in biopsies from the center or edge of the lesion and in the adjacent skin. Although the number of lepromatous cases was insufficient for statistical comparison, the mean densities of apoptosis observed in biopsies from the edge of established tuberculoid lesions (mean = 3.9 apoptoses/mm^2 granuloma) and lepromatous lesions (mean = 2.53 apoptoses/mm^2 granuloma)

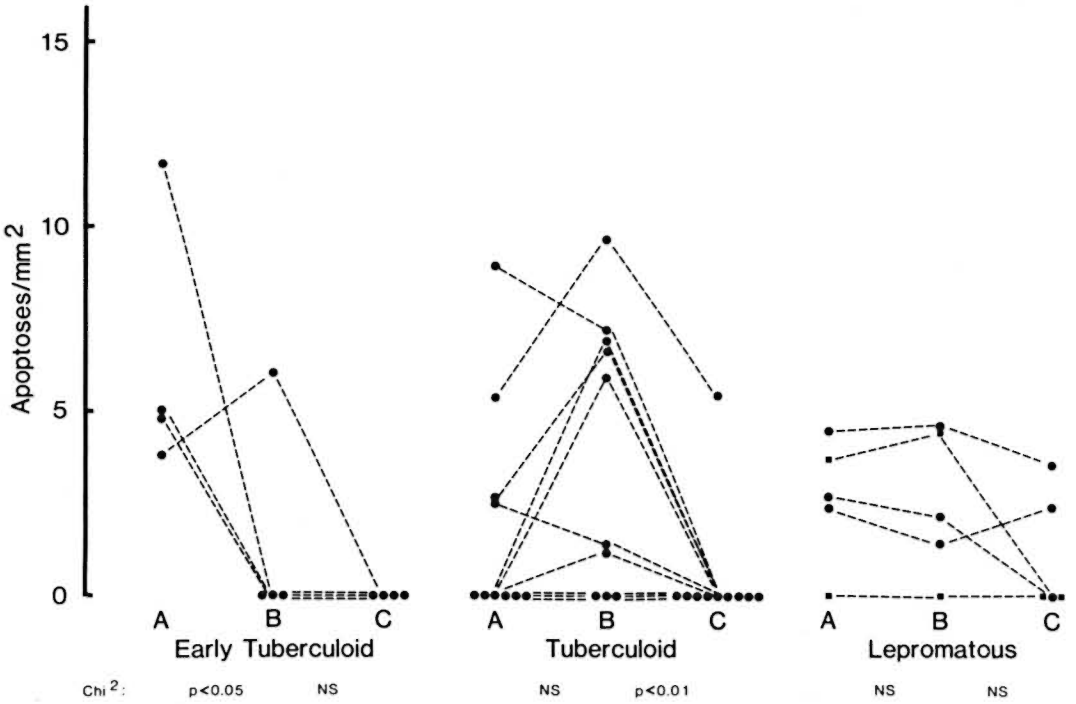

FIG. 3. Apoptotic counts for biopsies from the center (A), edge (B), and outside (C) of leprosy lesions. ■ = Results from cases classified as BL.

did not differ greatly. However, there appeared to be greater variability in the apoptotic count of the biopsies from the edge of tuberculoid lesions.

The mitotic counts were more variable in both tuberculoid and lepromatous lesions (Fig. 4). There is no overall pattern in either the lepromatous or the tuberculoid type of lesion. In tuberculoid lesions, mitoses were found in both the epithelioid cell and lymphocyte zones of the granulomas. Since lepromatous granulomas consist of a mixture of cell types, both lymphocytes and macrophages could have been dividing in these patients.

DISCUSSION

The results of the GF measurement confirm that biopsies taken from the edge of established lesions contain the greatest amount of granuloma and, therefore, are most suitable for diagnostic purposes ([2, 9]). However, the GF was greater in the central area in the four early BT lesions characterized by recognizable BT features in the central biopsy, but not at the edge. Some inflammation was present in the majority of the biopsies from skin outside the lesion. This varied from a mild mononuclear cell infiltrate around blood vessels in some of the tuberculoid cases, which may not have been due to the patients' leprosy, to established macrophage granulomas in the lepromatous cases. It is well known that lepromatous inflammation extends into clinically uninvolved skin in lepromatous leprosy ([3, 8]), and inflammatory changes have also been observed in skin outside tuberculoid lesions by a number of other authors ([3, 10, 11]).

We have observed apoptosis in leprosy granulomas of both the lepromatous and tuberculoid type. While it is not possible to identify with certainty the type of cell undergoing apoptosis, the situation of the apoptotic bodies in tuberculoid cases suggests that many of them were derived from epithelioid cells. In lepromatous cases, the apoptotic bodies could have been derived from any of the cell types present. They were easily distinguished from cells undergoing foamy degeneration. Apoptosis occurs over a relatively short time period (hours), and the finding of small numbers of apoptotic

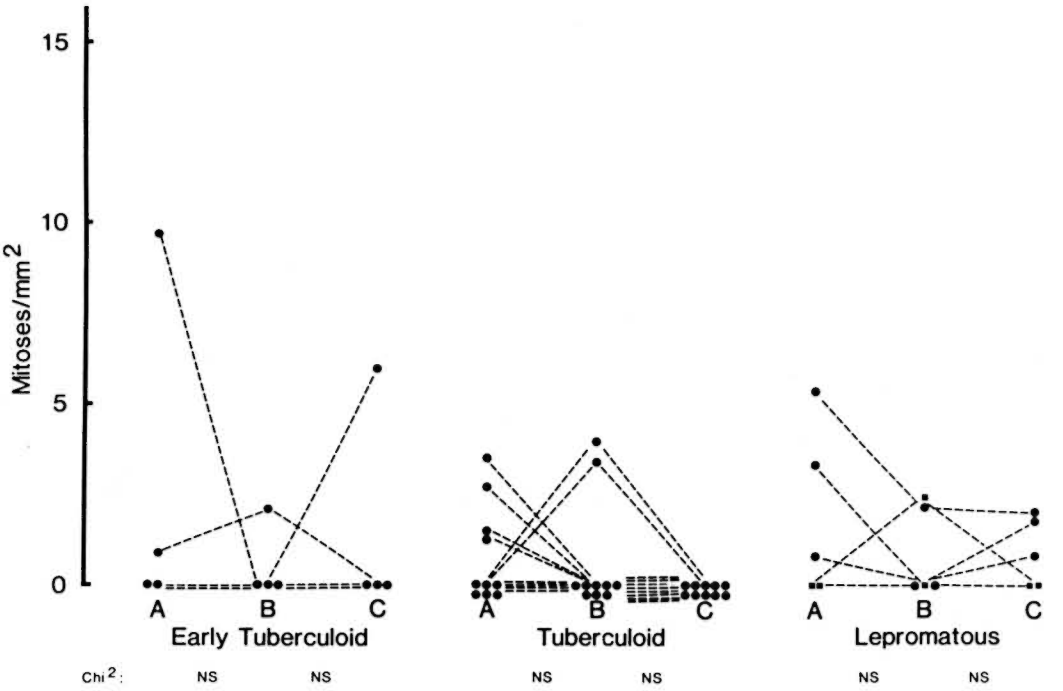

FIG. 4. Mitotic counts for biopsies from the center (A), edge (B), and outside (C) of leprosy lesions. ■ = Results from cases classified as BL.

bodies indicates that considerable cell loss is taking place ([4, 14]). The central biopsies from early tuberculoid lesions showed high rates of apoptosis; whereas established tuberculoid lesions have higher rates of apoptosis at their edges. There appears to be little difference in the small number of lepromatous lesions studied. This distribution of apoptosis is similar to that of the GF. Since resolution of experimental granulomas is associated with loss of epithelioid cells ([6]) and we have found apoptoses in the epithelioid cell zones of tuberculoid granulomas, cell death by this mechanism may be the principal factor in central healing of tuberculoid leprosy lesions.

The density of apoptotic bodies appears to be quite variable in tuberculoid lesions. From the results of this study, it is not possible to determine whether this variation is due to the position of the biopsy in relation to a gradient of apoptosis across the edge of tuberculoid lesions, or to individual variation in the patient's host response. However, it may prove possible to investigate the relationship of the apoptotic rate to histological and clinical activity in future studies.

Since counting apoptoses allows semiquantitative measurement of cell loss, we attempted to assess cell multiplication within the lesion in order to gain some understanding of cell turnover in leprosy lesions. In this preliminary study, it was practicable to count the number of mitoses present in the granulomas. In experimental granulomas, it is known that maintenance of epithelioid cell populations depends upon local cell division and the influx of new monocytes from the blood ([7]). The variability of our results may indicate that mitosis is less important than monocyte immigration from the blood in the maintenance of leprosy lesions. We are, therefore, developing methods for quantitating the influx of monocytes into leprosy lesions.

SUMMARY

We have studied the histological changes across leprosy lesions by taking biopsies from the center and edge of the lesions and from the clinically uninvolved skin outside

the lesions. A comparison of the granuloma fraction (GF) between biopsies from the center and edge of lesions and the adjacent unremarkable skin shows that the greatest GF is found at the edge of lesions, except in early tuberculoid (BT) cases when biopsies from the center have the greatest GF. Central healing of leprosy lesions occurs without tissue necrosis or appreciable fibrosis. Apoptosis, a form of individual cell death in living tissues, is known to be the mechanism of cell loss in a variety of situations, and we have found it to occur in leprosy lesions. Apoptotic activity is greatest at the edge of established tuberculoid lesions, but can be found in the center of the lesion in early cases. We, therefore, suggest that apoptosis is the mechanism by which epithelioid cells are lost during central healing in tuberculoid leprosy lesions. In the small number of multibacillary cases studied, apoptoses were found in biopsies from both the center and edge of the lesions. Mitoses can be found in biopsies from both lepromatous and tuberculoid lesions. However, the degree of mitotic activity does not appear to be related to the position of the biopsy within the lesion, and immigration of monocytes into the granulomas may be of greater importance than cell division in maintaining the numbers of epithelioid cells or macrophages present.

RESUMEN

Se estudiaron los cambios histológicos en las lesiones de todo el espectro de la lepra tomando biopsias del centro y del borde de las lesiones y de piel clínicamente no afectada fuera de las lesiones. La comparación de la fracción granuloma (FG) entre las biopsias del centro y borde de las lesiones y la piel normal, muestra que la FG mayor se encuentra en el borde de las lesiones, excepto en los casos tuberculoides tempranos (BT) donde las biopsias del centro tuvieron mayor FG. La curación central de las lesiones ocurrió sin necrosis tisular o fibrosis apreciable. La apoptosis, una forma de muerte celular individual en los tejidos vivos es un mecanismo de pérdida celular en una variedad de situaciones incluyendo las lesiones leprosas. La actividad apoptótica es mayor en el borde de las lesiones tuberculoides establecidas pero puede encontrarse en el centro de la lesión en los casos tempranos. Sugerimos que la apoptosis es el mecanismo por el cual se pierden las células epitelioides durante la regeneración central de las lesiones tuberculoides. En el pequeño número de casos multibacilares estudiados la apopteosis se encontró en las biopsias tanto del centro como del borde de las lesiones. Se pueden encontrar mitosis en las biopsias de los casos lepromatosos y tuberculoides. Sin embargo, el grado de actividad mitótica no parece estar relacionado con la posición de la biopsia dentro de la lesión, y la inmigración de monocitos en los granulomas puede ser más importante que la división celular en el mantenimiento del número de células epitelioides o de macrófagos presentes en las lesiones.

RÉSUMÉ

On a étudié les variations histologiques des lésions de lèpre en prélevant des biopsies au centre et à la périphérie des lèsions, de même que dans des fragments de peau sans signes cliniques en dehors de ces lésions. Si l'on compare la fraction granulomateuse (GF) dans les biopsies provenant du centre des lésions, de la périphérie de celles-ci, ou de la peau adjacente, on constate que cette fraction GF est plus importante à la périphérie des lésions; ceci n'est cependant pas le cas dans le lèpre tuberculoïde précoce (BT), où les biopsies prélevées au centre des lésions présentent la fraction granulomateuse la plus importante. La guérison centrale des lésions de lèpre survient sans nécrose tissulaire et sans fibrose appréciable. On sait que dans toute une série de situations, l'apoptose constitue un mécanisme d'élimination cellulaire; il s'agit de la mort de cellules individuelles au sein de tissus vivants. On a observé que ceci se produisait également dans les lésions de lèpre. L'activité apoptotique était plus prononcée à la périphérie des lésions tuberculoïdes bien établies; on n'a cependant pas pu l'observer dans le centre des lésions dans les cas précoces. On suggère dès lors que l'apoptose constitue le mécanisme par lequel les cellules épithélioïdes sont éliminées au cours de la guérison centrale des lésions de lèpre tuberculoïde. Parmi le petit nombre de cas multibacillaires qui ont été étudiés, des phénomènes d'apoptose ont été relevés dans les biopsies provenant tant du centre que de la périphérie des lésions. Les biopsies obtenues chez das malades lépromateux et tuberculoïdes présentaient à l'occasion des mitoses. Néanmoins, le degré d'activité mitotique ne semble pas être en relation avec l'endroit où la biopsie a été prélevée dans la lésion. La migration de monocytes dans les granulomes peut jouer un rôle plus important que la division cellulaire dans le maintien du nombre de cellules épithélioïdes ou de macrophages.

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