ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2009) 27: 147-152

Proliferation Index in Tuberculous Lymphadenitis

Pukpinya Jangjetriew and Sanya Sukpanichnant

SUMMARY During the pathogenesis of any granuloma, activated macrophages (AMs) are recruited and expanded under the influence of the migration inhibition factor. The goal of this study was to determine whether AMs then also proliferate by themselves or not. The proliferation index (PI) of AMs in lymph node biopsies from 40 cases of tuberculous lymphadenitis was evaluated by immunohistochemistry using a monoclonal antibody (MIB-1) to Ki-67. The PI was defined as the percentage of AMs with positive nuclear staining. It ranged from 3.6 - 20.6% (mean = 11.0 ± 4.4) which was slightly lower than that reported in previous studies. Mitotic figures ranged from 0 - 6 per 500 AMs. Multinucleated giant cells ranged from 0 - 6.3 cells per low power field and their PI was exclusively 0%. Areas of caseous necrosis ranged from 5-85% of the total area of the tissue section examined (mean = 61.6 ± 19.8). Mitotic figures, multinucleated giant cells, and areas of caseous necrosis lacked a statistically significant relationship to the PI ($p > 0.05$). In conclusion, AMs in granulomas can proliferate to a limited degree as detected by the mitotic figures and the PI.

A granulomatous reaction or "granuloma" can be found in infectious as well as non-infectious processes including some malignancies. In the case of malignancy, associated inflammatory cells are recruited to react with the neoplastic cells. This reaction itself may mask a malignancy whereas malignant cells may histologically mimic reactive inflammatory cells, in particular activated macrophages (AMs) or epithelioid histiocytes, making a distinction between malignant cells and AMs difficult.[1,2] This difficulty may lead to an incomplete diagnosis, especially when the malignancy is overshadowed by a granuloma, which again may lead to a delay of the proper treatment.

The pathogenesis of a granuloma is based on AMs being recruited from circulating monocytes and accumulated at the inflammatory site by the migration inhibition factor, after which they become activated and form a granuloma. These AMs are different from usual macrophages as they are increased in cell size due to an increased content of lysosomal enzymes. They show a more active metabolism and greater ability to kill ingested pathogens. In hematoxylin & eosin-stained (H&E) tissue sections they appear large, flat and pink; resembling squamous epithelial cells hence the name "epithelioid cells". Macrophages in chronic inflammatory sites can proliferate as they are not terminally differentiated[3] but AMs are different as they are activated macrophages with more activities as described above. Only a small number of AMs were reported to have the capacity to proliferate[4] so whether AMs can proliferate or not remains unclear. In contrast to the uncertainty surrounding the proliferation of AMs, malignant cells usually exhibit marked proliferation and have a high proliferation index (PI).

From the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.
Correspondence: Pukpinya Jangjetriew
E-mail: Drbanchong_jang@yahoo.com

The PI can be determined by the percentage of Ki-67 positive cells. The Ki-67 protein is an excellent marker to determine the growth fraction of a given cell population. It can be found in all active phases of the cell cycle (G_1, S, G_2, and mitosis), but is absent in resting cells (G_0).[5] MIB-1 is a monoclonal antibody (MAb) commonly used to detect Ki-67 and can be used in formalin-fixed paraffin-embedded tissue sections.[6,7] Only a few previous studies in the English literature reported a low PI of AMs in granulomas[4,8] and one report mentioned a low PI in reactive lymphadenitis.[9] In this study we hypothesize that in case of granulomas, the PI of AMs or any macrophages should be nil or very low. To test this hypothesis, tuberculous lymphadenitis, a prototype of a granulomatous disease, was used for determining the PI of AMs. The baseline data on the PI of AMs in tuberculous lymphadenitis should be useful for surgical pathology and understanding of the pathogenesis of granulomas.

MATERIALS AND METHODS

Lymph node tissue samples

Forty cases of tuberculous lymphadenitis as confirmed by the presence of acid fast bacilli (AFB+) were selected from the computerized database system of the Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, from January 2005 to December 2007. The inclusion criteria were established tuberculous lymphadenitis (AFB+) and adequate material for the study. Exclusion criteria were other causes of granulomatous lymphadenitis with negative AFB results, inadequate material for the study, and the presence of a malignancy in the lymph node biopsy.

The tissue blocks were retrieved and verified before sectioning. All histologic lymph node sections were reviewed to confirm the diagnosis of tuberculous lymphadenitis. One section for H&E staining and one for immunohistochemistry using MAb to Ki-67 (clone MIB-1, DAKO® code number M7240, Denmark) were performed for each case.

This study received approval from the Ethics Committee, Faculty of Medicine Siriraj Hospital, Mahidol University [No. Si016/2008].

Immunohistochemistry

Immunohistochemistry to determine the PI was performed the MIB-1 at a dilution of 1:600. After deparaffinization, the unstained tissue sections were put in the target retrieval solution, pH 6.0 (DAKO® solution, Denmark) at 95°C for 40 minutes, and at 26°C for 20 minutes. The endogenous peroxidase was blocked by incubating the section with 3% H_2O_2 in distilled water for 10 minutes. MIB-1 was then applied on the tissue sections as primary antibody. For secondary detection of the antibody binding, the Envision (HRP) kit (DAKO®, Denmark) was subsequently applied following the manufacturer's instruction. The end product of the antigen-antibody reaction was visualized using 3,3'-diaminobenzidine (DAB) as the substrate. The immunostained slide was then counterstained to visualize the nuclei with Carazzi hematoxylin, mounted with a coverslip, and histologically evaluated by the light microscopy.

Pathologic evaluation for PI and other histologic parameters

Five hundred nuclei of AMs per immunostained slide were examined for Ki-67 positive staining in the granulomatous areas corresponding to those found in the recut H & E sections (unequivocally brown stained nucleus) under a 40x objective lens using a microscopic grid. The PI was then calculated as the percentage of Ki-67 positive cells as follows: PI = (Number of Ki-67 positive nuclei/500 nuclei of AMs) x 100%.

Ki-67 positive activated lymphoid cells were not included in the counting. The cells could be differentiated since their nuclear features were different from those of AMs. The following histologic parameters were also evaluated: 1) mitotic figures per 500 AMs; 2) the number and Ki-67 positivity of multinucleated giant cells in each slide; and 3) the percentage of caseous necrosis areas compared to the total area of the tissue section examined. The data were collected in nominal scales and independently assessed by the two authors. All data were analyzed by descriptive statistics.

RESULTS

Forty lymph node biopsy samples reported in this study were obtained from 13 male and 27 fe-

male patients with a diagnosis of tuberculous lymphadenitis. Their ages ranged from 2-59 years (Mean ± SD = 32.1 ± 12.9 years). The sites of the lymph node biopsies included the neck (32 cases and 6 supraclavicular cases), axilla (1 case), and groin (1 case). The demographic information was obtained from the surgical pathology report of each case as approved by the Ethics Committee, Faculty of Medicine Siriraj Hospital, Mahidol University. There was no correlation between gender, age, sites of lymph node biopsy, and histopathologic findings of the cases.

Each of the 40 cases demonstrated granulomas containing many AMs, occasionally multinucleated giant cells (Fig. 1), and varied extent areas of caseous necrosis (Fig. 2). The Ki-67 positive AMs could be visualized clearly (Fig. 3). Their PI ranged from 3.6 to 20.6% (Mean ± SD = 11.0 ± 4.4) (95% confidence interval = 9.6-12.4). Mitotic figures ranged from 0 to 6 per 500 AMs. Table 1 shows the comparison between PI and mitotic figures. The first 14 cases (35%) showed mitosis, varying from 1-6 mitotic figures per 500 AMs while the other 26 cases (65%) did not show any mitotic figure among the AMs. The PI in the first 14 cases ranged from 3.6-17.8% while values in the other 26 cases ranged from 5.4-20.6%. Multinucleated giant cells ranged from 0-6.3 cells per low power field per slide and all showed negative Ki-67 staining (PI = 0%). No mitotic figure was detected in any multinucleated giant cell. The percentage of areas with caseous necrosis ranged from 5-85% (61.6 ± 19.8%) of the total area of the tissue section examined (Table 2). Mitotic figures, multinucleated giant cells, and caseous necrosis areas did not show any statistically significant relationship to the PI ($p > 0.05$).

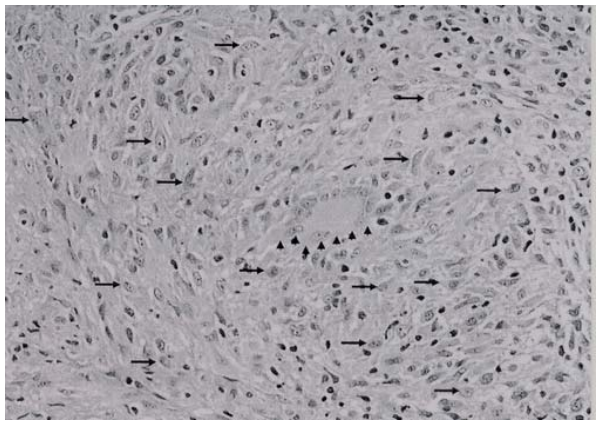

Fig. 1 Typical granuloma in tuberculous lymphadenitis. Many activated macrophages (arrows) and one multinucleated giant cell (arrow heads).

DISCUSSION

Information relative to the PI of AMs in granulomas is limited. Only few reports are currently available.[4,8] The histologic findings in this study demonstrated that while there were many AMs present within a granuloma, suggesting proliferation to a certain extent, the values of PI were predomi-

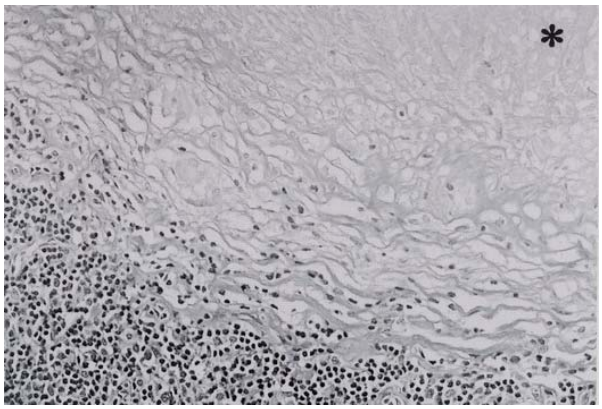

Fig. 2 Caseous necrosis area in the granuloma (*)

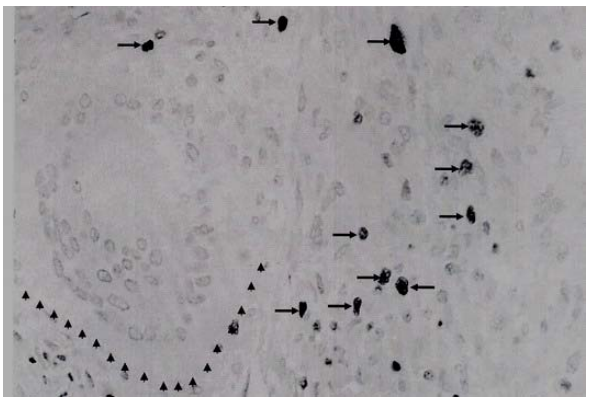

Fig. 3 Proliferation index (PI) demonstrated by Ki-67 positive cells. Note the positive dark nuclear staining of the nuclei of the activated macrophages (arrows), but negative staining of the nuclei of the multinucleated giant cell (arrow heads) (only nuclear counterstain shown).

nantly low as shown in Table 1. No mitosis was noted in 26 out of 40 cases (65%) and only 1 mitosis per 500 AMs in 9 cases (22.5%). Five cases (12.5%) showed 2-6 mitotic figures per 500 AMs. These AMs may be the result of the activation of macrophages that are recruited from circulating monocytes and accumulated at the inflammatory site by the migration inhibition factor and become an important cellular component of the granuloma.[3] It is not certain how well AMs can proliferate after the activation. Previous studies showed that only a small number of AMs can proliferate.[4,8] Valheim *et al.*[4] studied granulomatous lesions of subclinical intestinal paratuberculosis in goats and showed a very low PI measured by Ki-67 and most of the cells were recruited to the lesions. Chilosi *et al.*[8] used a double-marker technique (Ki-67 plus a macrophage-related marker) and found exceeding rare (1-2 per section) Ki-67 positive AMs in mediastinal lymph nodes of 2 out of 4 patients with sarcoidosis, a well-known disease with non-caseating granulomas. The finding of a low PI in AMs in sarcoidosis granulomas supports the results in the present study. Interestingly, 16 patients with sarcoidosis who had bronchoalveolar lavage (BAL) were found to have Ki-67 positive alveolar macrophages, varying from 2-14% (mean 6.5 ± 3.7%). The difference between the PI of AMs found in granulomas in the mediastinal lymph nodes and that of alveolar macrophages in the BAL samples raises the possibility of the different stages of macrophages as the reason for such a difference in PIs.[8]

The PI of AMs in the present study was lower than the PI of reactive lymphadenitis as reported by Schmitt *et al.*[9] (ranged, 3.6-20.6% *versus* 4-37%). In the present study, the histologic evaluation was performed in the tissue sections of lymph node biopsy diagnosed as tuberculous lymphadenitis. The Ki-67 positive cells could be determined whether or not they were AMs, based on morphology as well as the location of the cells. However, the study by Schmitt *et al.*[9] was performed on cytologic preparaions obtained from fine needle aspirates of the reactive lymph node. In such specimens the various cellular constituents of the lymph node are admixed. Hence in sections stained for Ki-67, it would be difficult to distinguish macrophages from centroblasts or activated lymphoid cells, both of which can be Ki-67 positive. Therefore, it would be very unlikely that the specific types of Ki-67 positive cells could be accurately determined in the reactive lymphadenitis cases described in the study by Schmitt *et al.*[9]

Table 1 Comparison between the proliferation index (PI) and mitosis per 500 activated macrophages (AMs) in 40 cases of tuberculous lymphadenitis

Case No.	PI (%)	Mitosis (%)
1	3.6	2 (0.4)
2	4.4	3 (0.6)
3	5.0	1 (0.2)
4	5.0	2 (0.4)
5	6.6	1 (0.2)
6	7.8	1 (0.2)
7	8.4	1 (0.2)
8	11.6	1 (0.2)
9	11.8	1 (0.2)
10	12.0	1 (0.2)
11	13.8	6 (1.2)
12	14.4	1 (0.2)
13	16.0	2 (0.4)
14	17.8	1 (0.2)
15	5.4	0
16	5.6	0
17	5.8	0
18	7.2	0
19	7.5	0
20	8.0	0
21	8.6	0
22	9.2	0
23	9.6	0
24	9.8	0
25	9.8	0
26	10.2	0
27	10.6	0
28	11.0	0
29	11.8	0
30	12.6	0
31	12.8	0
32	13.4	0
33	13.8	0
34	15.8	0
35	16.4	0
36	16.6	0
37	16.6	0
38	17.2	0
39	17.8	0
40	20.6	0

Table 2 Proliferation index (PI), areas of caseous necrosis, and number of multinucleated giant cells in tuberculous lymphadenitis

Parameter	Mean ± SD	Median (IQR)	Minimum	Maximum	95%CI
PI (%)	11 ± 4.4	10.8 (0.06)	3.6	20.6	9-12.4
Area of necrosis (%)	61.6 ± 19.8	65 (20.25)	5	85	**
Multinucleated giant cells*	9.6 ± 13.9	4 (13.5)	0	6.3	**

SD, standard deviation ; IQR, interquartile range (percentile 75 - percentile 25) ; CI, confidence interval; PI, proliferation index.
*First count per 10 low power fields then use the average per low power field.
**Previous studies for evaluation of 95% CI not available.

The present study failed to show a statistically significant relationship between the PI of AMs and other histologic parameters including mitotic rate, multinucleated giant cells, and areas of caseous necrosis. The reason underlying the lack of such a relationship is not known. Parenthetically, it is well accepted that the value of the PI is higher than the mitotic figures because Ki-67, the marker used to determine the PI, can be found in any cell entering the cell cycle,[5-7] while mitosis can be detected in histologic sections only in the mitosis phase of the cell cycle.

In addition, the present study demonstrated an interesting finding concerning multinucleated giant cells in granulomas. The formation of this particular cell is often described as a result of the fusion of many AMs.[3] The lack of mitosis and PI in multinucleated giant cells in this study seems to support the above hypothesis regarding the formation of these cells.

The finding of a low PI of the AMs in granuloma in the present study is applicable to routine practice in surgical pathology. When pathologists encounter with granulomatous reaction, it is important to decide whether it is infectious or non-infectious. Since tuberculous lymphadenitis is a prototype of granulomatous disease as well as a common infection in developing countries especially among immunocompromised patients, it is sometimes overdiagnosed and overtreated, especially in circumstances when the clinical findings favor tuberculosis, but acid fast bacilli cannot be demonstrated in the lesion. In many instances, malignancy can be obscured by the granulomatous reaction when the number of malignant cells are low. At times, the pathologic diagnosis may be rendered as "granulomatous inflammation, suspicious of tuberculosis," leading to a delayed diagnosis and the administration of anti-tuberculous drugs. Thus, pathologists should be aware of the possibility of malignancy when mitosis is detected in the granuloma because mitosis is not common in granuloma. In addition, pathologists should perform immunohistochemistry for Ki-67 to evaluate the PI of AMs in any granuloma when the clinical findings cannot distinguish between tuberculous lymphadenitis and malignancy since the PI of AMs is low in granulomas, but high in malignant cells.[5-9]

In summary, the results of the present study support the current view regarding the pathogenesis of granuloma in the literature as well as on the formation of multinucleated giant cells. In addition, the low PI and low number of mitoses of AMs in granulomas observed in the present study may assist pathologists in distinguishing between reactive processes and malignancy when a granulomatous reaction is present.

ACKNOWLEDGEMENTS

The authors greatly appreciate Mr. Suthipol Udompunturak's kind help with the statistical analysis. We also thank Ms. Kanittar Srisook for performing the immunohistochemistry. This work was supported by the Research Funding for Resident Research Projects from the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand. The authors also thank Prof. Dr. Fred Gorstein, the former Chairman of the Department of Pathology, Anatomy and Cell Biology at Jefferson Medical College, for his kind review and invaluable comments.

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