Journal of Korean Medical Science
Vol.3, No.3, P99-105, September 1988

Nucleolar Organizer Regions of Lymphomas in Korea

Insun Kim. M.D., Sung Hye Park, M.D., and Seung Yong Paik, M.D.

Department of Pathology, Korea University Hospital

Nucleolar organizer regions (NORs) are loops of DNA which occur in the nucleoli of cells and which possess ribosomal RNA (rRNA) genes. The numbers and/or configurations of NORs have been thought to be related to cellular activities. To assess the applicability of NORs associated protein (Ag-NORs) in the field of diagnostic histopathology, a silver staining was done in paraffin sections of malignant lymphomas, tonsils and reactive lymph nodes and the numbers of Ag-NORs in the nuclei of low-grade and those of high-grade lymphomas were compared. A significant difference was found between the numbers of Ag-NORs in the nuclei of low-grade lymphoma (a mean of 1.3 per nucleus) and those of high-grade lymphomas (a mean of 4.2 to 8.3 per nucleus). The Ag-NORs were often observed in nuclei in areas where nucleoli themselves were not visible in H&E stain. It is suggested that this method would be of great value in the field of tumor histopathology.

Key Words: *Nucleolar organizer regions (NORs), Silver stain, Malignant lymphomas.*

INTRODUCTION

There appears to be a growing interest amongst histopathologists about the role of the nucleus, DNA and cell kinetics and what investigations of these could be of value in the study of disease states. Evidence for this comes from the wealth of publications on 1) DNA flow cytometry, concerned with both DNA ploidy and cell proliferation(Barlogie et al., 1983), 2) the use of monoclonal antibody Ki67 which detects a proliferation-associated antigen (Gerdes et al.,1983), 3) the application of DNA and RNA in situ hybridization (Grody et al., 1987), and 4) studies of nucleolar organizer regions (Crocker & Nar, 1987 , Smith & Crocker, 1988).

Nucleolar organizer regions (NORs) are loops of DNA which are associated with secondary constrictions of the five acrocentric chromosomes and have been shown by in situ hybridization to be the site of the ribosomal RNA (rRNA) genes (Gall & Pardue, 1969). These latter are transcribed by RNA polymerase I (Perry, 1976) and are of vital significance in the ultimate synthesis of protein (Crocker & Nar, 1987). NORs have been studied for some years by cytogeneticists, who have made use of thier demonstration in the analysis of various genetic defects. The regions are readily demonstrated by means of an argyrophil technique (Ag-NORs) when applied to metaphase chromosome spreads (Howell & Black, 1980).

Their precise biochemical nature is unclear since they could consist of subunits of RNA polymerase I (Williams et al., 1982) or be a phosphoprotein of molecular weight 110KD, CD23 (Ochs & Busch,1984). Nonethless the argyrophilia of the NOR proteins acts as a marker of ribosomal RNA and possibly of its level of transcription (Miller et al.,1976, Morton et al.,1983) and could therefore provide useful information about the structure of the nucleolus and nucleolar activity. Thus the numbers and/or configurations of NORs may reflect the activities of cells in hyperplastic and neoplastic conditions.

A few recent studies showed that the reaction can be applied to routinely processed paraffin tissue sections (Ploton et al.,1986). To assess the applicability

Adress for Correpondence: *Insun Kim. M.D., Department of Pathology, Korea University Hospital, 2-4, Myungyun-dong, Chongro-ku, Seoul, Korea.*

of Ag-NORs in the field of diagnostic histopathology, silver staining was done in paraffin section of non-Hodgkin's lymphomas, tonsils and reactive lymph nodes and the numbers of Ag-NORs were compared in each cases.

MATERIALS AND METHODS

Thirty two spcimens were examined, from the same numbers of patients. These comprised the following classification of the working formulation of non-Hodgkin's lymphomas : Two cases of low grade lymphomas (one small lymphocytic and one intermediately differentiated lymphocytic), fourteen cases of intermediate grade lymphomas (two diffuse small cleaved, six diffuse, mixed small and large cell, two diffuse, large cell cleaved, two diffuse, large cell cleaved and noncleaved, two diffuse, large cell noncleaved), seven cases of high grade lymphomas (five large cell, immunoblastic, two lymphoblastic) and two cases of miscellaneous group (one malignant histiocytosis, one plasmacytoma). In addition, two histologically normal palatine tonsils and five lymph nodes exhibiting reactive hyperplasia were studied.

In all cases, the tissues had been fixed in 10 percent buffered formalin and processed to paraffin wax.

STAINING TECHNIQUE

Sections of 5μm thickness were cut from the routinely processed paraffin blocks. These were dewaxed in xylene (3-5 min), and then rehydrated through ethanols to distilled water. The Ag-NOR staining solution was prepared by dissolving gelatin in 1 percent aqueous formic acid at a concentration of 2 percent. This solution was then mixed, 1:2 volumes, with 50 percent aqueous silver nitrate solution, to give the final working solution. This was immediately poured over the tissue sections and left for 60 min at room temperature. The silver solution was then washed off with distilled water and the sections were dehydrated through graded ethanols to xylene and mounted in medium.

COUNTING PROCEDURE

In all specimens, 100 cells of each case were examined using a ×100 oil immersion objective to a total magnification of ×1000. The tumor cells were taken at random and the numbers of Ag-NOR dots per each cell were counted. The range and mean numbers of Ag-NORs from each case were determined. In addition, the proportion of cells having different numbers of Ag-NORs per nucleus were expressed as percent. In reactive lymph nodes and palatine tonsils, the numbers of Ag-NORs were counted from the following cells ; interfollicular lymphocytes, mantle zone lymphocytes, centrocytes and centroblasts and high endothelial venule (HEV) cells, and in non-Hodgkin's lymphomas, the Ag-NOR numbers were counted in the malignant cells of lymphomas.

RESULT

In all specimens, clearly defined silver stained dots were observed in all nuclei. It was observed in many cell types that there was not necessarily a relationship between the position of hematoxylin-stained nucleoli or chromatin and Ag-NORs. For example, in classical immunoblastic lymphoma, with a solitary central nucleolus, there were usually multiple Ag-NORS.

1. Palatine tonsils

The pharyngeal and crypt epithelia possessed more Ag-NORs in their basal areas (mean 2.3) than superficial cells (mean 1.0) with a gradual change between them. The high endothelial venules possessed only approximately one Ag-NOR per nucleus (mean 1.0) as did interfollicular lymphocytes (mean 1.0) and mantle zone lymphocytes (men 1.0) and follicular centrocytes (mean 1.4). However, centroblasts in lymphoid follicles contained significantly more Ag-NORs (mean 3.9) than the previous lymphoid cell.

2. Reactive lymph nodes

Similar results were found for Ag-NOR numbers in the cell types common to lymph nodes and tonsils : high endothelial vessels (mean 1.0) ; interfollicular lymphocytes (mean 1.0) ; mantle zone lymphocytes (mean 1.0) ; centrocytes (mean 1.3) ; centroblasts (mean 4.8) (Fig. 1).

3. Non-Hodgkin's lymphomas

The high-grade lymphomas possessed significantly far more Ag-NORs than did low-grade ones. Low grade lymphoma, small lymphocytic (mean 1.3, range 1 to 4), intermediately differentiated lymphocytic (mean 2.5, range 1 to 5), intermediate-grade lymphomas, diffuse small lymphocytic (mean 2.5, range 1 to 4), diffuse mixed small and large cell (mean 3.3, range 1 to 12), diffuse large cell (mean 3.3, range 1 to 12), diffuse large cell cleaved (mean 3.6, range 1 to 9), dif-

fuse large cell cleaved and noncleaved and large cell noncleaved (mean 8.3, range 3 to 20) and high-grade lymphomas, immunoblastic (mean 4.2, range 1 to 12) and lymphoblastic (mean 5.6, range 1 to 12). The mean Ag-NORs of the miscellaneous group lymphomas, malignant histiocytosis and plasmacytoma were 4.4 (range 1 to 11) and 3.7 (range 1 to 8), respectively. These data are summarized in Fig. 2-11.

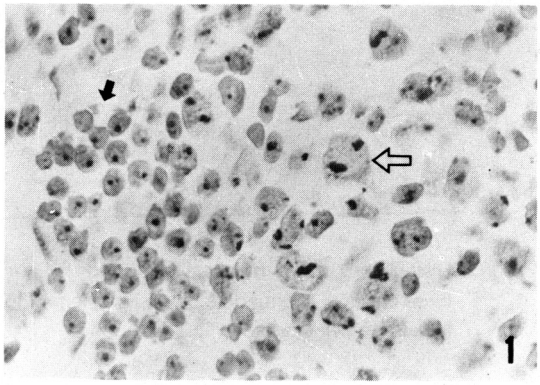

Fig. 1. Reactive lymph node: The left half of the field shows mantle zone lymphocytes containing average one Ag-NOR in the center of the nuclei (black arrow). The right half is lymphoid follicular center which contains small and large follicular center cells showing multiple Ag-NORs (open arrow). (Silver stain, ×1000)

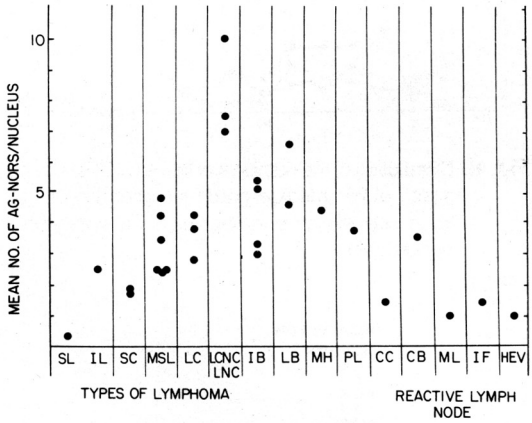

Fig. 2. Distribution of mean numbers of Ag-NORs in each cases of malignant lymphomas and normal cells in reactive lymph nodes, (SL; small lymphocytic, IL. Intermediately differentiated lymphocytic, SC: small cleaved, MSL; Mixed small and large cell, LC; large cell cleaved, LCNC; large cell cleaved and noncleaved, LNC; Large all noncleaved, IB; Immunoblastic, LB; Lymphoblastic, MH; Malignant histiocytosis, PL; Plasmacytic, CC; Centrocytes, CB; Centroblasts, ML; Mantle zone lymphocytes, IF; Interfollicular lymphocytes, HEV; High endothelial venule.

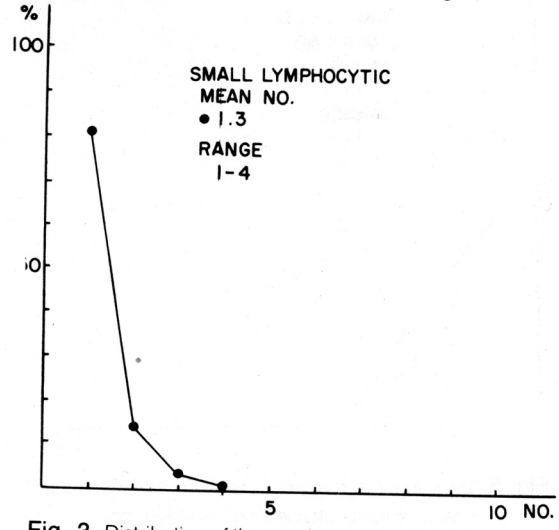

Fig. 3. Distribution of the numbers of the Ag-NORs in one case of small lymphocytic lymphoma.

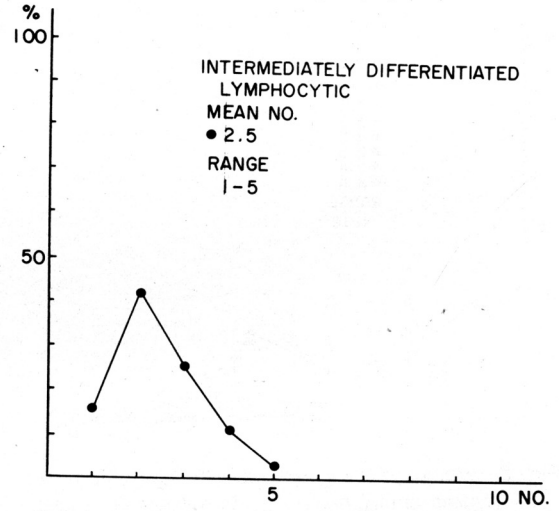

Fig. 4. Distribution of the numbers of the Ag-NORs in one case of intermediately differentiated lymphocytic lymphoma.

The range of numbers and configurations of Ag-NORs were different between each case. The large numbers of nuclei of small lymphocytic (82%) and small cleaved lymphomas (50%) had one Ag-NOR of which configurations showed regular sized round shape and their ranges were 1 to 4 and 1 to 5, respectively. In mixed small and large cell lymphoma, the large cells possessed far more Ag-NORs than small cells which had one or two. The large cell noncleav-

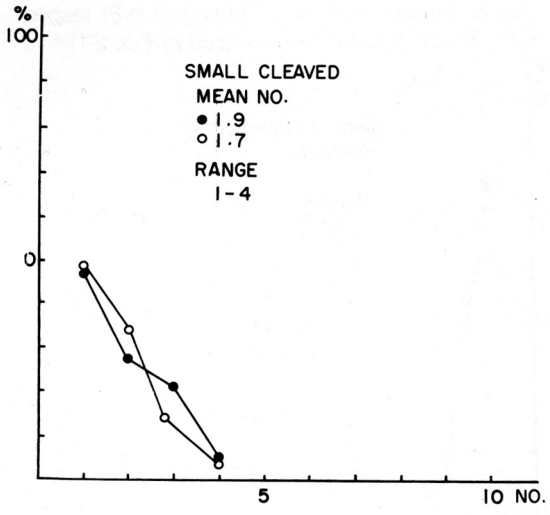

Fig. 5. Distribution of the numbers of the Ag-NORs in two cases of small cleaved cell lymphomas.

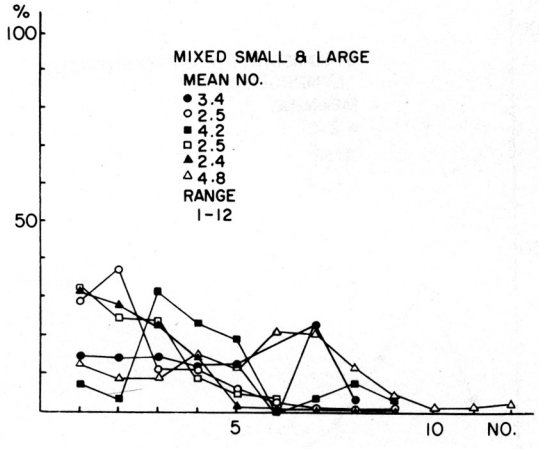

Fig. 6. Distribution of the numbers of the Ag-NORs in six cases of intermediate grade lymphoma, diffuse mixed small and large cell type.

ed lymphomas had the most large numbers of Ag-NORs. The immunoblastic lymphoma had more Ag-NORs (mean 4.2) than one nucleolus showed in H&E section and their configurations were large round or small round. The lymphoblastic lymphomas revealed fine dot-like multiple Ag-NORs (Fig. 12-17).

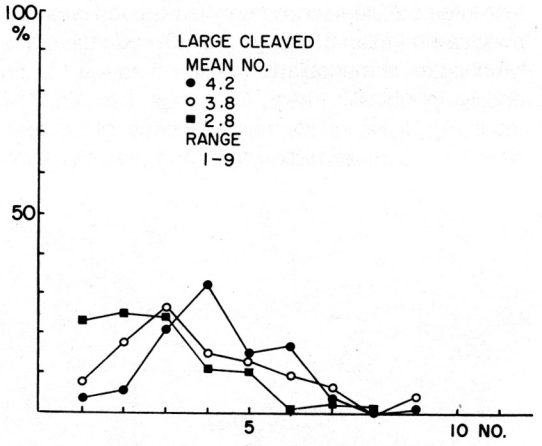

Fig. 7. Distributions of the numbers of the Ag-NORs in three cases of intermedate grade lymphoma, diffuse large cell cleaved.

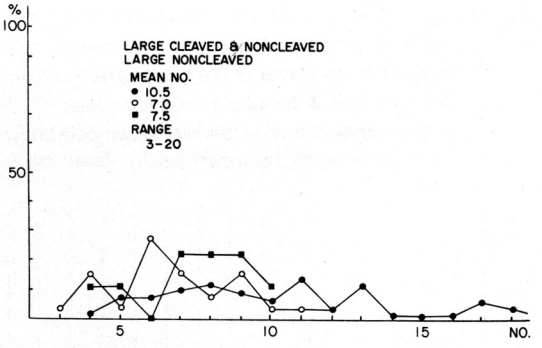

Fig. 8. Distribution of the numbers of the Ag-NORs in three cases of intermedate grade lymphomas, diffuse large cell cleaved and noncleaved and large cell noncleaved.

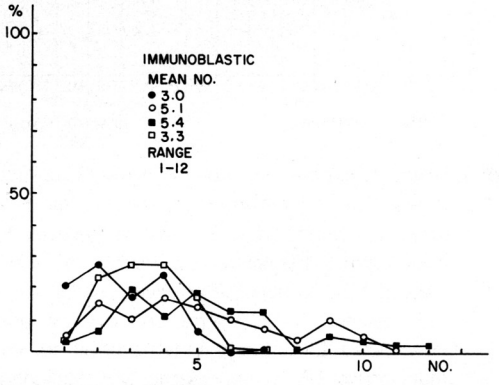

Fig. 9. Distribution of the numbers of Ag-NORs in four cases of high grade lymphomas, large cell immunoblastic.

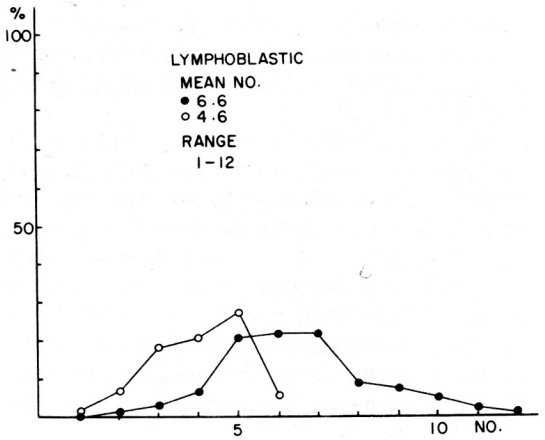

Fig. 10. Distribution of the numbers of Ag-NORs in miscellaneous group, malignant histiocytosis and plasmacytoma.

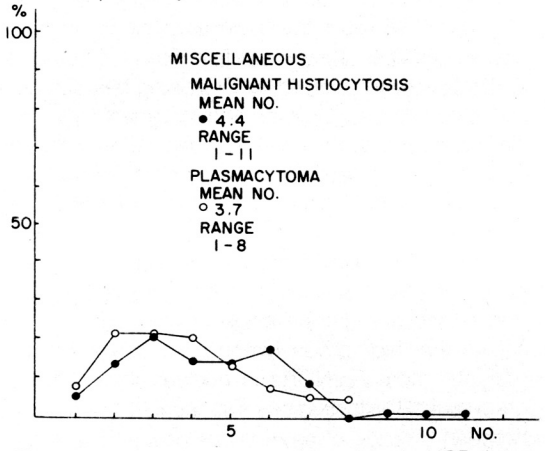

Fig. 11. Distribution of the numbers of the Ag-NORs in two cases of high grade lymphoma, lymphoblastic.

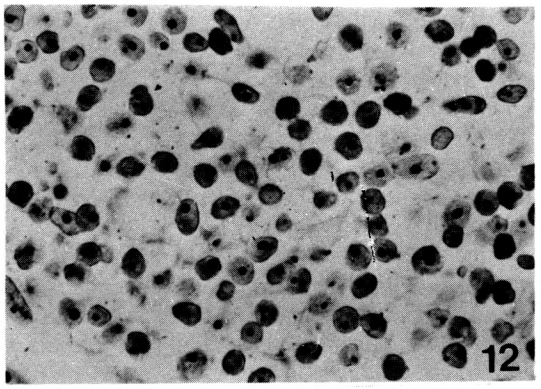

Fig. 12. Low grade small lymphocytic lymphoma: Most of the cells have one Ag-NOR in the nucleus. (Silver stain, ×1000).

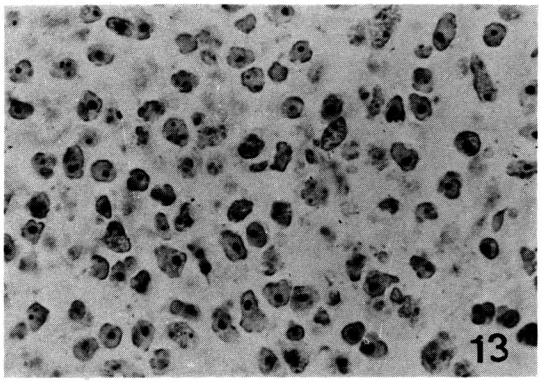

Fig. 13. Intermediate grade, diffuse, small cleaved cell lymphoma : Most of the cells have one Ag-NOR and some of the cells contain multiple Ag-NORs (Silver stain, ×1000).

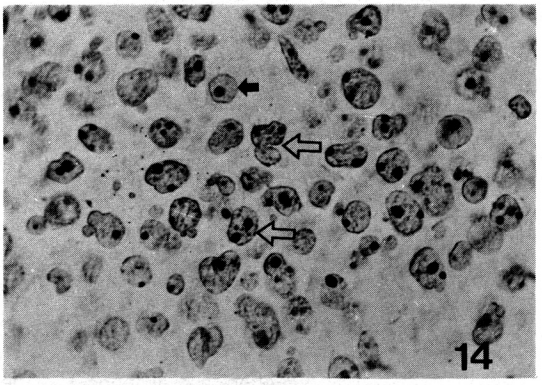

Fig. 14. Intermediate grade, diffuse, mixed small and large cell lymphoma : The small cells contain one Ag-NOR (black arrow) whereas the large cells have two or more Ag-NORs (open arrow). (Silver stain, ×1000).

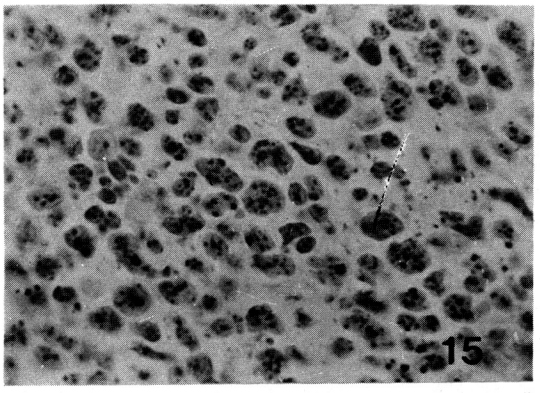

Fig. 15. Intermediate grade, diffuse, large noncleaved cell lymphoma : Most neoplastic cells have numerous small Ag-NORs. (Silver stain, ×1000).

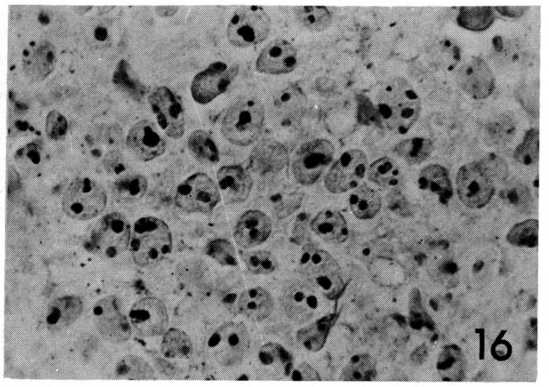

Fig. 16. High grade, large cell immunoblastic lymphoma : The neoplastic cells have one or multiple large Ag-NORs. (Silver stain, ×1000).

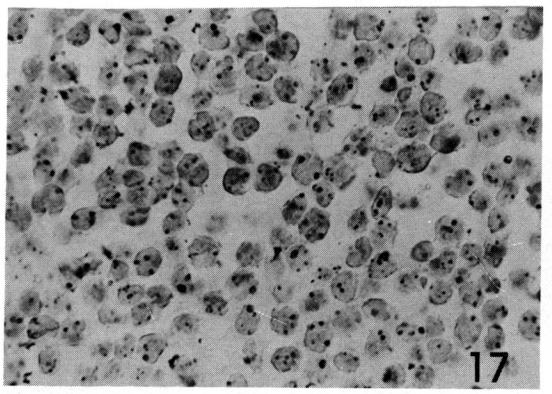

Fig. 17. High grade, lymphoblastic lymphoma : The neoplastic cells show mutiple fine dot-like Ag-NORs, (Silver stain, ×1000).

DISCUSSION

Non-Hodgkin's lymphomas morphologically and immunologically are a heterogenous group and accurate histological diagnosis is often difficult, but histologic classification is important as it clearly has clinical relevance. The differentiation of the various cellular components of lymphomas usually depends upon the recognition of characteristic nuclear morphology and the distribution of chromatin and nucleoli. The Giemsa stain may be useful than hematoxylin-eosin statin in the evaluation of the nuclear morphology.

Silver stained NOR sites (Ag-NORs) have been studied in man for over a decade (Goodpasture & Bloom, 1975, Howell et al., 1975), by means of the so-called Ag-NOR technique. By means of this methods, Ag-NORs may be seen on the short arms of five acrocentric human chromosomes, namely 13,14,15,21 and 22.

The role of NORs is uncertain. Two possible molecules thought to be NOR-associated proteins include RNA polymerase itself (Williams et al., 1982) and CD23 protein (Ochs & Busch, 1984). The latter has a molecular size of 110 KD and there is also a 100 KD protein associated with NORs, which is quite similar to CD23 protein (Escande et al., 1985, Gas et al., 1985). Both can be identified immunologically. An 80 KD nucleolar protein has also recently been described, by means of immunoblotting of nuclear proteins.

Recently the staining method was modified and enable much more rapid staining by means of a onestep method (Howell & Black, 1980). Silver binding was observed in the nuclei as black dot. In 1981, Kacerovska et al. showed a difference in the numbers of dot-like structures on Ag-NOR staining between normal, stimulated, suppressed and malignant cells. This was confirmed by Busch et al., More recently, Ploton et al. in 1986 showed numerous dots in the nuclei of HL60 promyelocytic leukemic cells and K562 promyeloblastic cells.

In human malignant tissues, Crocker and Nar used the Ag-NOR staining at room temperature with paraffin sections of non-Hodgkin's lymphomas. It was shown that high-grade lymphomas contained significantly more Ag-NORs in nucleus than did low-grade ones. Distinction has also recently been made between a range of nevocellular nevi and a variety of melanoma, as well as between hyperplastic and neoplastic breast lesions on the basis of Ag-NOR numbers (Crocker & Skilreck, 1987, Fallowfield et al., 1988). Thus it appeared that a quantitative and semi-quantitative examination of Ag-NORs could render an indication of the status of cells with regard to activation, induction or malignancy.

In the current study, it has been shown that, in reactive lymphoid tissue, most component cells, with the exception of follicular centroblasts, possess approximately one Ag-NOR per nucleus. Many such cells are 'resting' with division in only a fairly small compartment : these latter cells could account for mean Ag-NOR counts in slight excess of unity. Centrobleasts possess a greater number of Ag-NORs than other lymphoid follicle center cells. Likewise, the more active basal cells of the epithelium of the tonsils possess more Ag-NORs than the upper, less active squamous cells. These findings appear to be reflected in the specimens of non-Hodgkin's lymhoma, where those

of a low-grade small lymphocytic lymphoma possess only one and fewer Ag-NORs than those of large cell and lymphoblastic lymphoma.

In the other intermediate grade types, the number of Ag-NORs were intermediate between low and high grade lymphomas.

It must be mentioned that the Ag-NOR technique should not be regarded as being a method which demonstrates nucleoli. The chromatin or nucleolar patterns obtained with hematoxylin-eosin or Giemsa staining are often different to those observed on Ag-NOR staining. For example, in typical immunoblastic lymphomas which possessed one large central nucleolus on conventional staining, several Ag-NORs were noted.

The value of quantitative study in this field has previously been emphasized and it has been demonstrated that, in non-Hodgkin's lymphoma of high-grade, measurements of nucleoli themselves, with regard to size, number and eccentricity, may provide useful morphometric data.

It will be of great interest to investigate the Ag-NOR reaction with enumeration, in relation to other techniques, such as DNA flow cytometry and labelling with proliferating cell markers such as the antibody Ki 67. Furthermore, clinical correlations must also be examined.

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