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CHROMOSOMAL PROTEINS IN PULMONARY ALVEOLAR MACROPHAGES

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ABSTRACT

In this study the nature of chromosomal proteins, histones and nonhistone in resident alveolar macrophages was investigated in comparison to peritoneal neutrophils and calf thymus proteins. Cells were obtained by lavaging the lung and after purity determination they were subjected to fractional extraction procedures. Proteins were then analysed on SDS polyacrylamide gels and densitometric scans were obtained. The results show that in macrophages, the pattern of histone and nonhistone proteins were similar to thymus proteins, two distinct and specific proteins H1° and HMG14b and also a protein with a molecular weight of 12kd (Hx) were present. These data suggest a different chromatin protein pattern in pulmonary alveolar macrophages.

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INTRODUCTION

In eukaryotic cells, chromatin is composed of DNA, histones, nonhistones and a small amount of RNA[1]. Histones are basic proteins divided into five main fractions named H1, H2A, H2B, H3 and H4.[2] These proteins are widely distributed and participate in the nucleosomal structure of chromatin.[3] Between them histone H1 is involved in the coiling of nucleosomes into higher order structures.[4] This protein is highly heterogeneous, composed of a family of isoprotein species differing in their primary structure.[5,6] The high mobility group (HMG) class of chromatin proteins are among the most abundant and ubiquitous nonhistone proteins found in the nuclei of all higher eukaryotes. These proteins also consist of four proteins HMG1, 2,14 and 17.[7-9] It has now been accepted that these proteins participate in genome function such as replication and transcription.[10]

Our previous experiments have shown that the dense chromatin of neutrophilic granulocytes have specific HMG protein patterns in which 80% reduction in HMG1 content was observed and HMG14 and H1° were completely absent.[11] Pulmonary alveolar macrophages are cells of the phagocytic mononuclear system that are considered as terminally differentiated cells although still active in protein synthesis. These cells participate in many aspects of host defence against bacteria and tumors,[12] therefore the purpose of the present study was to examine the characteristics of chromosomal proteins in macrophages in comparison with those in neutrophils and thymus.

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MATERIALS AND METHODS

Albino rats of either sex and weighing 100-350g were used throughout the experiments. They were purchased from Hessarak Institute in Karaj. Calf thymus was obtained from Ziaran slaughter house and frozen immediately in liquid nitrogen before use.

Alveolar macrophages were collected from rat lungs using the bronchoalveolar lavage procedure described before.[13] Lungs were lavaged thirty times each with 10-15ml saline and at least five times infusion. In each experiment 10-15 rats were lavaged. The cells were collected by centrifugation at 2000g and after examination of the intactness and purity they were frozen in liquid nitrogen until use. On test day the cells were thawed, mixed and homogenized in 5% perchloric acid. The amount of DNA was determined by measuring absorbance at 260nm. Proteins were extracted three times and then subjected to fractional acetone precipitation procedure.[14] Core histones were also extracted from residual chromatin by acid and ethanol or acetone precipated.[15]

Proteins were electrophoresed on SDS-polyacrylamide gels as described by Lammeli,[16] with some modifications. Stacking gel was 4%, separating gel 15% and TCA fixation was omitted. Molecular weight standards (Sigma) of 10000-70000 D were run parallel to the samples. Densitometric scanning of the stained bands were performed using Beck R-112 gel scanner and the quantity of the proteins determined by calculating the area under the peaks.

The amount of proteins were estimated by Lowry method modified by Hartree.[14] Bovine serum albumin was used as a protein standard.

RESULTS

Fig.1 illustrates the number of macrophages obtained from rat lungs belonging to three different weight ranges. It is shown that rats weighing higher than 270g were suitable for this purpose giving about $5\text{-}8\times10^6$ cells per rat lung. Cytological examination of the cytospin slides indicated that 95-98% of the cells were intact macrophages. Although most early studies of phagocytosis focused on peritoneal macrophages because of their accessibility, pulmonary alveolar macrophages thus obtained represented a relatively homogeneous population.

SDS-polyacrylamide gel electrophoresis pattern and densitometric scans of histone H1 extracted from alveolar macrophages in comparison to thymus H1 is given in (Fig. 2). It is shown that H1 from macrophages resolved into three main bands but in thymus only two bands belonging to H1A and H1B was observed. The

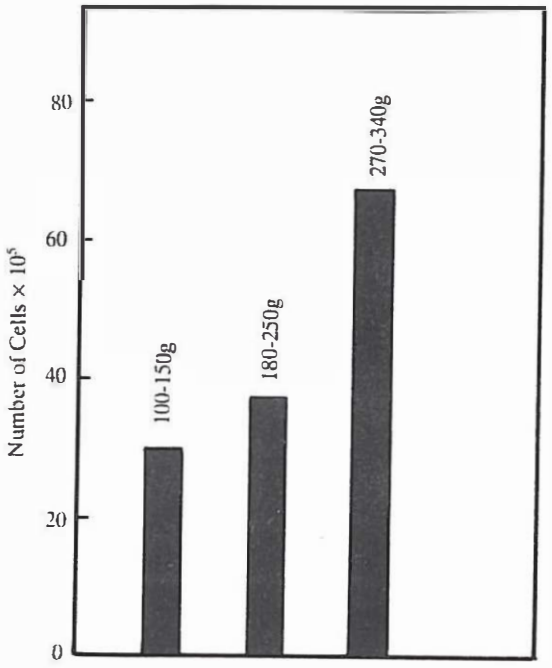

Fig.1. Number of alveolar macrophages obtained from different weights of rats (20 separate lavages).

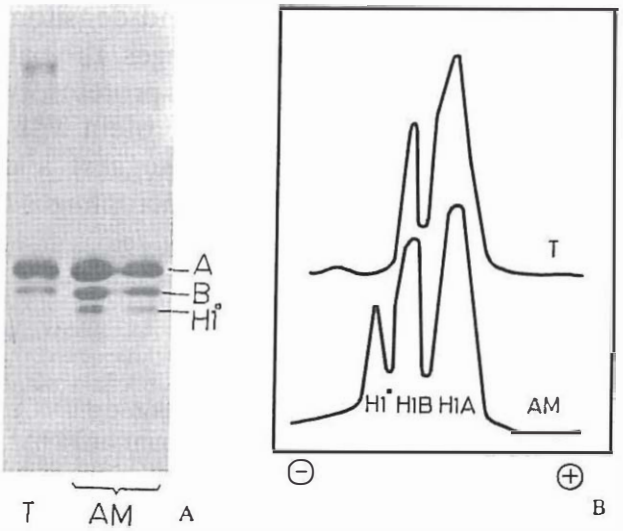

Fig.2. SDS Polyacrylamide gel and densitometric scan of histone H1 from: T: Thymus, AM: Alveolar Macrophages (20 and 10 ug loaded).

third band in macrophage gel pattern resemble $H1^0$, a subtype of H1 in specialized cells. The densitometric scanning pattern (Fig. 2B) also confirmed the results. Analysis of the core histones from acid extracted material of macrophages, neutrophils and thymus on the gel was carried out and the density of the bands were determined. Fig. 3 shows the results. In macrophages, apart from four histones similar to thymus core histones, the main difference was a protein with a mobility

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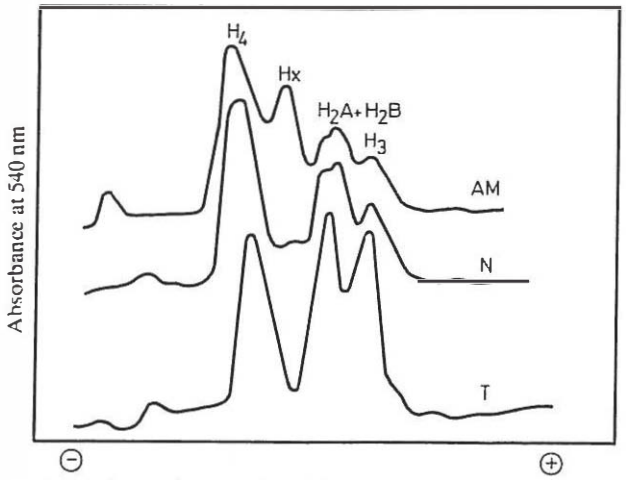

Fig.3. Densitometric scans of core histone proteins from: T; Thymus, AM; Alveolar macrophages, N; Neutrophils.

running ahead of histone H4. This protein was called Hx and gave a molecular weight of 12kd when measured against standard proteins.

Nonhistone chromosomal proteins extractable with perchloric acid (HMG proteins) were similar between thymus and macrophages (Fig. 4) but were different from neutrophil proteins. Also, in macrophages, HMG14showed two bands named 14a and 14b while in thymus it appears in a single band and in neutrophils it is completely absent.

Table I summarises the quantity of the proteins

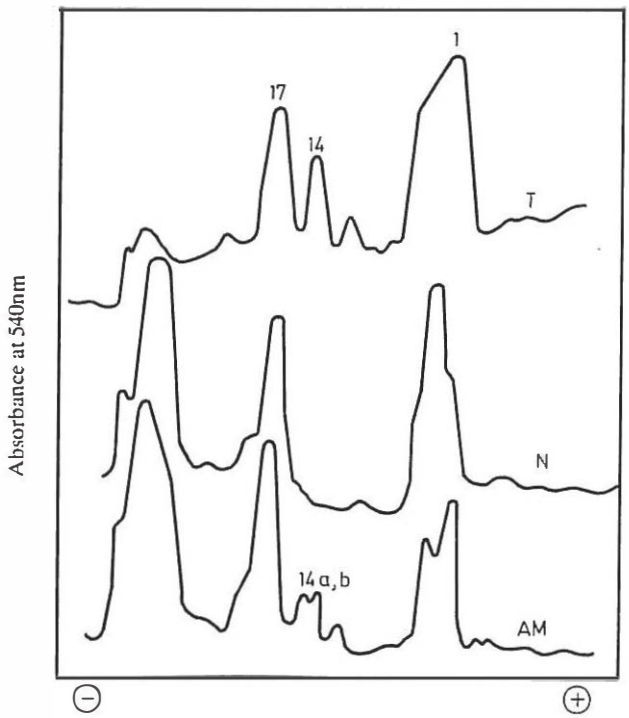

Fig.4. Densitometric scans of HMG nonhistone proteins from T; Thymus, AM; Alveolar macrophages, N; Neutrophils.

Table I: Comparison of the quantity of the special proteins in three different cellular types.

Sample	Area mm²				
	$H1^0$	Hx	HMG14a	HMG14b	Ub.
Macrophages	9.05	7.09	3.2	2.9	20.4
Neutrophils	ND	ND	ND	ND	20.6
Thymus	ND	ND	5.7	ND	3.7

ND: Not detected
Number of experiments were five to seven separate experiments.

appearing in macrophages in comparison to two other sources studied. The quantity was determined by measuring the area under the peaks. It is seen that apart from differences in $H1^0$, Hx and HMG14, macrophage, and neutrophil cells contained higher content of ubiquitin (a protein found in conjugated form with H2A and H2B[18] in chromatin) than in thymus.

DISCUSSION

We have described here the characteristics of chromosomal proteins in alveolar macrophages and have shown some defined differences between these cells with those of neutrophilic granuloctyes and thymus. Pulmonary alveolar macrophages play an important role in lung defence mechanisms against inhaled pathogens as well as inert irritants.[19] These cells are considered differentiated cells which only undergo cell division under appropriate conditions using mitogens or other stimuli.[20] The present results indicate that preparation of histone H1 from whole cells or nuclei of macrophages contain H1A, H1B and $H1^0$ subtypes which have been tentatively identified by their solubility in acid, molecular weight value and electrophoretic behavior. Subfractions of H1A and H1B are normaly present in all H1 preparations (in a high quantity) from a wide variety of tissues or single cells, but a main difference is observed in $H1^0$ which is restricted to nondividing cells.[21] In addition the correlation of $H1^0$ with the hormonally dependent functional activity of the cells from several rat and mouse tissues has been reported.[22] In spite of these observations, data presented by Banchev, et al[23] indicate that while H1A and H1B are associated with bulk chromatin, $H1^0$ is compartmentalised in some chromatin regions only. These regions might contain the transcriptionally active genes.

Our results which indicate the presence of $H1^0$ in macrophages but not in fully differentiated neutrophilic granulocytes are possibly correlated with the latter observation. Macrophages in normal state, are nondividing cells but still are active in protein synthesis,[12]

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therefore $H1^0$ may be connected with special parts of chromatin and function as a regulator in some cellular processes. It is also clear that histone H1 plays a central role in forming the higher order structure of chromatin.[4,24] Various H1 subfractions show different ability to condense chromatin fragments.[5] All these data suggest that histone H1 family are part of the molecular mechanisms responsible for the genome function.

Macrophages also show two distinct sets of proteins, Hx and HMG14b. Although they are suggested to be a modified form of histone H4 and HMG14 respectively, the nature and function of them is still unknown.

In conclusion resident alveolar macrophages with their extensive function represent a special set of chromosomal proteins. One way to find out further information about these proteins is to look at the mRNA level in these cells, work which is now under investigation.

ACKNOWLEDGEMENTS

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