Changes of Histone H1 Subtypes with Aging in Strains of Mice That Possess Different Immunological Characteristics

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Relative proportions of histone H1 proteins were determined for brain, heart, liver, and spleen for five strains of mice as a function of age. The strains examined were SJL/J and MRL/MPJ-lpr/lpr which develop early resistance to tolerance and A/J, C57BL/6J and MRL/MPJ-+/-+ which do not. Heart, brain, and liver of most of these strains displayed significant relative increases in histone H1o and coordinate decreases in H1' or H1" with age. In contrast, spleen cells, which are highly proliferative, contained little or no histone H1o. Only spleen cells from a mouse strain with a predisposition to lupus erythematosus, MRL/MPJ-lpr/lpr, displayed any significant H1 changes.

Key Words: Chromatin, Linker histones, Mouse tissues

Data from experiments with rat and mouse lend support to the view that in adult life transcription decreases with increasing age (Richardson et al., 1983). Taken together with the observed increases in certain of the linker histones with age (Medvedev et al., 1977, 1978), a cause and effect relationship may be postulated. In other words, either a general age-related increase of these linker proteins or a change in the relative proportions of the subtypes might result in some of the chromatin shifting to a more condensed or heterochromatized condition. This increased chromatin condensation might nonspecifically inhibit transcription. In fact, there is a recent report of decreased susceptibility of mouse brain neuronal chromatin to nuclease digestion with increasing age (Berkowitz et al., 1983).

As a first step toward determining whether a relationship exists between changes in the H1 proteins and transcription, a systematic study of the relative levels of these linker proteins in a variety of different mouse tissues as a function of age was undertaken. Earlier investigations have been restricted to a single strain or few tissue types. In the present study five strains of mice were examined. The strains selected were among those that have been shown previously to differ significantly in immunological parameters such as the rate of regulatory T cell changes with age (Cinader, 1983; Hosokawa & Cinader, 1983). Thus we can measure lymphoid linker histone proportions with functional aging of the immune system and attempt to correlate those results with linker histone levels in other tissues for which our only aging parameter is
the chronological age of the animal relative to its average life expectancy.

Among the strains, SJL/J was selected because it serves as a prototype for strains that develop resistance to tolerance (Nakano & Cinader, 1980a). To this group belong MRL/MPJ-lpr/lpr, and we have also included its congenic partner, MRL/MPJ-+/+, which can be tolerized at a more advanced age than animals with the lpr/lpr gene (Amagai & Cinader, 1981). Mice of genotype lpr/lpr develop massive lymph node enlargement, hypergammaglobulinemia, autoantibody formation, and, ultimately, immune complex glomerulonephritis. Inbred congenic mice of strain MRL/MPJ offer opportunities for the analysis of lpr gene effects on the development of autoimmune disorder. Mice of genotype Ipr/lpr differ significantly from mice of genotype +/+ and also from SJL animals (Amagai & Cinader, 1981) in their response to normally tolerogenic molecules. Mice of strain A/J and C57BL/6, which are our long-lived controls, were also examined because they show a relatively slow decrease in suppressor capacity (Amagai et al., 1982; Nakano & Cinader, 1980b), compared with the very rapid progression found for some of the other strains mentioned above.

MATERIALS AND METHODS

Experimental animals and tissues. — The mice (females, 5 weeks old) from strains A/J; C57BL/6J, SJL/J; MRL/MPJ-lpr/lpr and MRL/MPJ-+/+ were purchased from Jackson Laboratory (Bar Harbor, ME) and allowed to age in our own colony. All animals were housed in groups of five in plastic cages (30 × 18 × 12 cm) at 23 °C. A contact bedding, BetaChips, was used and changed twice a week. The mice were kept on a 12 hour light/dark cycle. Purina Laboratory Rodent Chow 5001 and water were provided ad libitum. All animals were checked daily for disease or health problems; unhealthy animals (e.g., skin lesions, tumors) were excluded from the study. Skin scratches were examined for ectoparasites, and bacterial swabs were taken when deemed necessary. Procedures such as necropsy or microbiological examination were not employed routinely. The colony was not maintained behind a barrier. After the sacrifice of animals of different ages and strains, the liver, heart, brain, and spleen were carefully removed, placed directly on dry ice, and then stored at -70 °C until further use.

The mean life span for SJL/J mice is 395 ± 11 days (n = 76) (Strorer, 1969), 512 ± 9 days (n = 59) for A/J and 695 ± 9 days (n = 99) for C57BL/6J (Russel, 1966). The autoimmune strains MRL/MPJ-lpr/lpr and MRL/MPJ-+/+ have a 50% mortality at 5 and 17 months, respectively (Andrews et al., 1978).

**Linker protein extraction.** — Frozen tissue pooled from 5 mice of one particular strain and age was homogenized in 5% (v/v) perchloric acid, 0.5 mM phenylmethylsulfonylfluoride with a Polytron tissue homogenizer. The homogenate was centrifuged at 1100g for 30 min. The resulting sediment was extracted once again in the same way. The combined supernatants were clarified by filtration through a sintered glass funnel. The solution was adjusted to 18% trichloroacetic acid (w/v), left overnight at 4 °C and the resulting precipitate collected. This precipitate was washed once with acidified acetone (0.2% HCl), twice with dry acetone, and vacuum dried. Just prior to electrophoretic analysis the histones were first dissolved in 10 mM HCl and then buffer was added.

**Electrophoretic analysis.** — Linker histone protein samples were resolved on 15% SDS (Laemmli, 1970) slab gels (1.5 mm × 19 cm × 16 cm long). After electrophoresis, the gels were stained with Coomassie Blue R, destained and scanned at 530 nm with a Canalco Model G densitometer interfaced to an Apple computer and an Interactive Microware ADALAB digitizer.

The entire track was scanned from the origin for every sample. For the histone H1 bands about 250 independent data points were taken in a 15 mm distance. Areas under protein peaks were determined by numerical peak detection and integration. From four to eight gel tracks were scanned for each tissue sample for a particular age and strain. Each of these tracks were on separate gels. One slab gel was used to compare histones for different tissues with age for a particular strain. The data was averaged, the standard deviation calculated, and t tests were applied to determine whether statistically significant changes had occurred.

RESULTS

Densitometer traces of a Coomassie stained slab gel and a photograph of the gel itself, typical of the type obtained in this study, are shown in Figure 1. The three histone bands from left to right are denoted as I, II, and 0. Histone H1 contains subtypes b, d, e; H1n subtypes a, c; and H1n subtypes a, b as resolved by two dimensional SDS, acid-urea gel
Brain, heart, and liver. — The results of a quantitative analysis of more than 250 gel tracks of histone H1 proteins extracted from brain, heart, and liver from three ages of five strains of mice are shown in Figure 2. The results are expressed as a percentage of the total H1 histone. Standard deviations indicated by error bars for the proportions of the three H1 fractions were in most cases less than 3% and in all cases less than 8%. The chronological age of mice used in an analysis for a particular strain represent young adult, middle-aged adult, and old adult with reference to the average life span for that strain.

The results shown in Figure 2 were analyzed by t tests. The histone H1 changes that exceeded 99% confidence limits for a null hypothesis in at least two values in a set of three were judged as statistically significant. In almost all cases only two of the three histone subtypes changed with increasing age. For the changes that were statistically significant, the ratios of the two affected histone H1 types with increasing age are shown in Figure 3. We found for brain, heart, and liver that in most cases histone H1° increased with age, whereas H1′ decreased. Exceptions are a decrease of H1° in brains from mouse strains A/J and MRL/MPJ-lpr/lpr and H1° constancy in A/J heart.

Spleen. — Histone H1 extracted from mouse spleen contains very little histone H1° as can be seen in Figure 2. Earlier studies have also reported this histone H1° deficit in mouse spleen (Lennox & Cohen, 1983). In contrast with brain, heart, and liver we observed no significant changes in the proportion of H1′ and H1° subtypes with increasing age in mouse spleen. A striking exception occurred in MRL/MPJ-1pr/1pr, which showed a significant increase in histone H1′ at the expense of H1° with increasing age.

DISCUSSION

Age-dependent changes observed in histone H1 subtypes in mouse brain, heart, and liver, although small were nevertheless reproducible and statistically significant. They are in agreement with earlier qualitative studies that reported an increase in H1° with age in some mouse tissues (Medvedev et al., 1977, 1978). For the different tissues examined, the results shown in Figure 3 indicate the existence of some strain specific changes. For example in A/J brain histone H1° decreases whereas in MRL/MPJ-
cells such as brain and heart which have five- to tenfold higher H1\textsuperscript{a} levels, spleen histone H1 proportions remain invariant with age. An exception to this occurs in mouse strain MRL/MpJ-1pr/1pr, which displays major functional changes of the immune system with age. It may be that the observed increase in H1\textsuperscript{a} and concomitant decrease in H1\textsuperscript{a} in spleen from this strain has a direct connection to these functional changes. We note that only in A/J and MRL/MpJ-1pr/1pr brain does H1\textsuperscript{a} decrease, but in these H1\textsuperscript{a} remains constant with increasing age. Thus the change in the relative proportions of H1\textsuperscript{a} and H1\textsuperscript{a} is unique to spleen cells of the MRL/MpJ-1pr/1pr strain and clearly deserves further attention.

We have developed a variety of strategies by which the progression of functional changes in the immune system can be altered (Cinader, 1983), and we intend to apply these in the future to examine the possibility that there are linker histone changes in spleen cells that correlate with functional changes.

Figure 3. Ratio of histone H1 subtypes displaying statistically significant changes in brain, heart, liver and spleen with increasing age in adult mice. The vertical axis shows the ratios, the horizontal axis the age in weeks and strain as shown, below that line. The particular histone H1 subtypes in a ratio is indicated above each bar graph group.

+/- H1\textsuperscript{a} decreases, histone H1\textsuperscript{a} increases in both. No significant H1 changes are found for SJL/J heart nor for A/J and MRL/MpJ-1pr/1pr liver. The lack of observed change might arise from the fact that the histone H1 bands as resolved on SDS gels are composed of several components (Lennox & Cohen, 1983). Thus in an apparently invariant situation, one of the subfractions might increase whereas another decreases. For example subtype a in H1\textsuperscript{a} might decrease whereas subtype c increases, resulting in no observable change in the area due to H1\textsuperscript{a}. Quantitative analyses of two-dimensional electrophoretic separations are required to settle this question.

Because only a small fraction (less than 5%) of the mouse genome is expressed in any one cell type, even the small changes observed here in the H1 fractions could have a major effect on the transcriptional competence of a gene if they occur in a transcriptionally active domain of chromatin. Whether or not the additional H1\textsuperscript{a} binds to chromatin that was transcriptionally competent remains to be established and forms the basis of some of our future studies. In this regard, we have shown earlier that histone H1\textsuperscript{a} is preferentially associated with a nucleosome resistant fraction of chromatin isolated from the brains of individuals afflicted with Alzheimer’s disease (Lewis et al., 1981) where a decrease in transcription of neuronal chromatin is suspected.

The fact that spleen cells lack H1\textsuperscript{a} and are highly proliferative is intriguing. It seems that, in contrast with tissues composed of slowly or nondividing

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