Chromosoma (Berl.) 74, 51–55 (1979)

CHROMOSOMA

Sister Chromatid Exchange in Murine Alveolar Macrophages, Regenerating Liver and Bone Marrow Cells – A Simultaneous Multicellular in vivo Assay

Mary K. Conner, Yves Alarie and Rebecca L. Dombroske

Departments of Industrial Environmental Health Sciences and Human Genetics, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, U.SA.

Abstract. Differential labeling of sister chromatids was achieved simultaneously in murine alveolar macrophages, regenerating liver, and bone marrow cells of partially hepatectomized mice as well as in alveolar macrophages and bone marrow cells of nonhepatectomized mice. The mean frequency of SCE/cell $\pm$S.D. and the percentage of second division cells for each cell type were determined. No significant differences in mean frequencies of SCE/cell were observed among the cell types or between hepatectomized (alveolar macrophages – 3.6 ± 2.2, bone marrow – 3.4 ± 2.2; regenerating liver – 3.6 ± 2.4) and nonhepatectomized (alveolar macrophages – 3.4 ± 1.9; bone marrow – 2.9 ± 1.8). Although the percentage of second division cells was dependent upon cell type, no significant differences were apparent between hepatectomized (alveolar macrophages – $57 \pm 8\%$; bone marrow – $37 \pm 6\%$; regenerating liver – $65 \pm 6\%$) and nonhepatectomized mice (alveolar macrophages – $53 \pm 6\%$; bone marrow – $36 \pm 4\%$). Comparisons between BrdU treated and nontreated nonhepatectomized mice revealed no significant alteration in mitotic yields.

Introduction

In vivo BrdU labeling of chromosomes for demonstration of sister chromatid exchange (SCE) promises to be a valuable means of evaluating cytogenetic toxicity of chemical agents whose activities depend upon host alteration (Allen and Latt, 1976; Vogel and Bauknecht, 1976). Investigation of specific cellular accessibility and susceptibility is facilitated by the availability of techniques for effecting SCE in murine bone marrow (Allen and Latt, 1976; Schneider et al., 1976; Vogel and Bauknecht, 1976), spleen (Allen et al., 1977) spermatogonia (Allen and Latt, 1976) and, most recently, regenerating liver cells (Conner et al., 1978). Since relative cellular sensitivities depend on tissue residence and metabolic capability, analysis of induced SCE in a variety of critical tissue cells, harvested simultaneously from the same animal, is expected to further enhance the value of this in vivo assay.

0009-5915/79/0074/0051/$01.00

The mammalian lung occupies a unique position as a portal of entry between the organism and its environment and, as such, is directly susceptible to mutagenic/carcinogenic action of hazardous airborne agents. Furthermore, a rich pulmonary vascular blood supply makes it a target for many indirectly acting mutagens. In recent years, the lung has also gained recognition as an organ of importance in the metabolism, disposition and/or accumulation of xenobiotic agents (Philpot et al., 1977; Hook and Bend, 1976).

The most readily accessible (by pulmonary lavage) and, therefore, most extensively studied lung cell is the alveolar macrophage which is normally adherent to the alveolar walls where it serves as a major defender against foreign materials, including particulate matter. In the present investigation, we describe a method for simultaneously effecting SCE in murine alveolar macrophages, regenerating liver, and bone marrow cells of hepatectomized mice, as well as in alveolar macrophages and bone marrow cells of nonhepatectomized mice. Baseline SCE levels are presented for each cell type.

Materials and Methods

All mice used in this study were 3 month old BDF_1 males (27–32 g) from a newly established breeding colony of parental strains, females C57Bl/6J and male DBA/2J.

DNA Labeling. Mice were subjected to 2/3 partial hepatectomy between 11:00 a.m. and noon. On the fifth day post hepatectomy, hepatectomized and nonhepatectomized mice were given 9 hourly injections (0.2 ml each) of BrdU (20 mg/2.2 ml saline/mouse) beginning at 3:30 p.m. through 11:30 p.m. At 7:30 a.m. the following day, each mouse was given an i.p. injection of 100 μg of colchicine, followed by sacrifice by cervical dislocation at 11:30 a.m. Liver (from hepatectomized mice only) and/or bone marrow cells were isolated, harvested, and stained as previously described (Conner et al., 1978).

Alveolar Macrophage Isolation. Following removal of the femurs and liver, the trachea and lungs were exposed by careful excision of the rib cage. A cannula was inserted, via an incision, into the trachea and tied securely. The trachea and lungs were then excised from the chest cavity and dissected free of the thymus and heart. Following removal of external blood by briefly dipping the lungs in water, they were placed in a funnel such that the cannula extended beyond the stem of the funnel which in turn was supported in a rack above a beaker. The lungs were fully inflated (approximately 2.5 ml) with calcium and magnesium free Hank's solution (pH 7.4, 37°C) from a 10 cm^3 syringe attached to the cannula via a 20 gange needle. The syringe was supported over the beaker and the lungs left inflated until the lungs from all of the mice were excised and inflated. The lungs were then drained, massaged gently and reinflated. This process was repeated using a total of 20 ml of Hank's solution. Subsequent treatment of cell suspension and preparation of slides were identical to that of bone marrow (Conner et al., 1978).

Analysis of Slides. In order to establish baseline values, the described simultaneous cell harvests were performed several times over a period of 6 weeks and include a total of 10 hepatectomized and 10 nonhepatectomized mice. For each cell type from each animal, 30 cells were scored for number and distribution of SCE. In addition, 100 cells of each type were examined for percentage of second division cells.

A semiquantitative comparison of alveolar macrophage mitotic yields from 6 BrdU treated and 6 nontreated nonhepatectomized mice was performed by counting the total number of metaphases on the first of four slides prepared from each animal. Relative cell densities were estimated by counting the total number of cells and metaphases in 12 corresponding, randomly chosen fields (40× objective). If necessary, additional corresponding, randomly chosen fields were counted to obtain an estimate of the total number of metaphases present in 1,000 cells.

Results

Following sacrifice at 20 h after the first of 9 hourly BrdU injections, differential labeling of sister chromatids was achieved simultaneously in alveolar macrophages, regenerating liver, and bone marrow cells of partially hepatectomized mice as well as in alveolar macrophages and bone marrow cells of nonhepatectomized mice. The mean frequency of SCE/cell ± S.D. and the percentage of second division cells for each cell type are presented in Table 1. No significant differences in mean frequencies of SCE/cell were oberserved among the cell types or between hepatectomized (alveolar macrophages – 3.6 ± 2.2; bone marrow – 3.4 ± 2.2; regenerating liver – 3.6 ± 2.4) and nonhepatectomized mice (alveolar macrophages – 3.4 ± 1.9; bone marrow – 2.9 ± 1.8). Although the percentage of second division cells was dependent upon cell type, no significant differences were apparent between hepatectomized (alveolar macrophages –

Table 1. Mean SCE/cell ± S.D. (scored in 30 cells) and percentage second division cells (scored in 100 cells) in alveolar macrophages, bone marrow and liver cells of hepatectomized mice and alveolar macrophages and bone marrow cells of nonhepatectomized mice

Animal	Alveolar macrophages		Bone marrow		Liver	
Hepatectomized:	Mean SCE/cell ± S.D.	% 2nd division cells	Mean SCE/cell ± S.D.	% 2nd division cells	Mean SCE/cell ± S.D.	% 2nd division cells
H_1	4.5 ± 2.7	58	4.5 ± 3.3	26	3.2 ± 2.6	62
H_2	4.5 ± 2.4	73	2.2 ± 1.4	35	4.4 ± 1.6	65
H_3	4.3 ± 3.2	65	4.2 ± 2.3	42	4.1 ± 3.7	73
H_4	3.9 ± 2.1	62	2.6 ± 2.0	45	3.1 ± 2.2	70
H_5	3.1 ± 2.3	54	3.7 ± 2.1	26	3.5 ± 2.5	66
H_6	2.6 ± 1.3	45	4.2 ± 2.5	39	3.7 ± 3.0	58
H_7	3.5 ± 1.1	58	3.2 ± 2.0	44	3.2 ± 1.5	70
H_8	3.4 ± 1.5	53	3.6 ± 1.8	38	3.8 ± 2.1	70
H_9	3.2 ± 1.5	55	3.0 ± 2.0	36	3.5 ± 2.2	64
H_{10}	3.1 ± 1.6	59	2.6 ± 1.7	36	3.5 ± 2.1	54
	3.6 ± 2.2	57 ± 8	3.4 ± 2.2	37 ± 6	3.6 ± 2.4	65 ± 6
Nonhepatectomized:						
N_1	3.4 ± 1.7	57	2.6 ± 1.3	32		
N_2	3.3 ± 2.0	49	2.1 ± 1.2	32		
N_3	3.8 ± 1.6	64	3.4 ± 2.0	40		
N_4	3.9 ± 1.8	43	3.0 ± 1.8	35		
N_5	3.0 ± 2.0	49	2.9 ± 1.5	30		
N_6	3.4 ± 1.8	52	2.5 ± 1.6	42		
N_7	3.5 ± 2.4	54	4.3 ± 2.8	42		
N_8	3.7 ± 2.4	59	2.5 ± 1.4	37		
N_9	3.2 ± 1.6	55	3.3 ± 1.8	33		
N_{10}	2.9 ± 1.6	53	2.3 ± 1.2	38		
	3.4 ± 1.9	53 ± 6	2.9 ± 1.8	36 ± 4		

Table 2. Total metaphase yield; total number of cells in 12 corresponding randomly chosen fields (40 × objective); and number of metaphases/1,000 cells as determined on the first alveolar macrophage slide from each mouse. (Each group includes 6 nonhepatectomized mice)

	Total number metaphases/slide mean (range)	Total number cells/12 fields mean (range)	Metaphases/1,000 cells mean (range)
BrdU treated	144 (44–271)	617 (460–933)	0.7 (0–3)
No BrdU	118 (19–226)	707 (229–1,030)	2.2 (0–5)

$57 \pm 8\%$; bone marrow – $37 \pm 6\%$; and regenerating liver – $65 \pm 6\%$) and nonhepatectomized mice (alveolar macrophages – $53 \pm 6\%$; bone marrow – $36 \pm 4\%$).

Comparisons were made between BrdU treated and nontreated hepatectomized mice to determine whether BrdU incorporation significantly affects the mitotic yield of alveolar macrophages. Because the relatively low mitotic indices of alveolar macrophage preparations were found to vary considerably among animals, slides, and regions of the same slide, semiquantitative comparisons of mitotic yield and relative cell densities were made. The total number of metaphases counted on the first alveolar macrophage slide from each animal, the total number of cells counted in 12 corresponding, randomly chosen fields (40 × objective), and the number of metaphases/1,000 cells (determined in corresponding fields) are presented in Table 2. Incorporation of BrdU does not appear to significantly alter the mitotic yield or relative cell density of macrophage preparations.

Discussion

Despite the relatively low mitotic indices in alveolar macrophage preparations from BrdU treated as well as nontreated mice, sufficient mitotic yields were consistently obtained for assessment of percentage of second division cells in 100 metaphases and SCE scoring in 30 metaphases per mouse. Such metaphase yields are in contrast to chromosomal studies of radiation chimeras which, in spite of macrophage stimulation by ether and Freund's adjuvant, produced a maximum of only 15 mitoses/animal (Pinkett et al., 1966). Besides the possible deleterious effect of radiation on mitosis, several variations incorporated into our harvest procedure may explain the substantially higher metaphase yields. Whereas divalent calcium and magnesium ions appear to play a critical role in adhesion of alveolar macrophages to alveolar walls, the presence of these cations has been implicated as the cause of decreased total cell yields from lungs (particularly of small rodents) lavaged with balanced salt solutions as compared to physiological saline (Brain et al., 1977). Serial washings with saline and gentle massage of the lungs also resulted in more efficient cell lavage (Brain et al., 1977). It is not surprising, therefore, that increased mitotic yields should also result from combined massage and serial washes using calcium and magnesium free Hank's solution.

The alveolar macrophage cell cycle time observed in this study (2 cycles in 20 h) compares favorably with that of 9.2 h, estimated from ^{3}H-thymidine uptake in NO_2 stimulated macrophage production (Evans et al., 1973). Although the apparent similarities in cycling characteristics of alveolar macrophages, regenerating liver, and bone marrow are suggestive of their potentially common hematopoietic origins (Van Furth, 1970), such rapid cycling times are more consistent with local proliferation of lung and liver cells rather than migration and maturation of bone marrow precursors. Application of BrdU labeling kinetics in this multicellular system should permit complete evaluation of local tissue macrophage stimulation and marrow recruitment following exposure to chemical agents.

Perhaps the greatest value of this multicellular assay is simultaneous assessment of relative mutagen induced effects in a variety of critical tissues. By virtue of their different tissue residences, alveolar macrophages, regenerating liver, and bone marrow cells are expected to reflect metabolic and functional capabilities unique to their positions in specified organs.

Acknowledgements. This research was supported under Research Grant No. 2 RO1-OH 00367 from the National Institute of Occupational Safety and Health and in part from the Mobay Chemical Fellowship in Toxicology.

References

Allen, J.W., Latt, S.A.: In vivo BrdU-33258, Hoechst analysis of DNA replication kinetics and sister chromatid exchange formation in mouse somatic and meiotic cells. Chromosoma (Berl.) **58**, 325–340 (1976)

Allen, J.W., Schuler, C.F., Mendes, R.W., Latt, S.A.: A simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromodeoxyuridine tablets. Cytogenet. Cell Genet. **18**, 231–237 (1977)

Brain, J.D., Sorokon, S.P., Godleski, J.J.: Quantitation, origin and fate of pulmonary macrophages. In: Lung biology in health and disease, Vol. 5: Respiratory defense mechanisms, II. (J.D. Brain, D.F. Proctor and L.M. Reid, eds.), pp. 849–926. New York: Marcel Dekker 1977

Conner, M.K., Boggs, S.S., Turner, J.H.: Comparison of in vivo BrdU labeling methods and spontaneous sister chromatid exchange frequencies in regenerating murine liver and bone marrow cells. Chromosoma (Berl.) **68**, 303–311 (1978)

Evans, M.J., Cabral, L.J., Stephens, R.J., Freeman, G.: Cell division of alveolar macrophages in rat lung following exposure to NO_2. Amer. J. Path. **70**, 199–205 (1973)

Hook, G.E.R., Bend, J.R.: Minireview: Pulmonary metabolism of xenobiotics. Life Sci. **18**, 279–290 (1976)

Philpot, R.M., Anderson, M.W., Eling, T.E.: Uptake, accumulation and metabolism of chemicals by the lung. In: Lung biology in health and disease, Vol. 4: Metabolic functions of the lung. (Y.S. Bakhle, and J.R. Vane, eds.), pp. 123–172. New York: Marcel Dekker 1977

Pinkett, M., Cowdrey, C.R., Nowell, P.C.: Mixed hematopoietic and pulmonary origin of "alveolar macrophages" as demonstrated by chromosome markers. Amer. J. Path. **48**. 859–867 (1966)

Schneider, E.L., Chaillet, J.R., Tice, R.R.: In vivo BrdU labelling of mammalian chromosomes. Exp. Cell Res. **100**, 396–399 (1976)

Van Furth, R.: Origin and Kinetics of Monocytes and Macrophages. Sem. in Hematol. **7**, 125–141 (1970)

Vogel, W., Bauknecht, T.: Differential chromatid staining by in vivo treatment as a mutagenicity test system. Nature (Lond.) **260**, 448–449 (1976)

Received March 30, 1979/Accepted April 18, 1979 by J.H. Taylor
Ready for press May 7, 1979