of the L-chain gene is kept constant despite the massive synthesis of DNA during the 5th instar implies that the expression of the L-chain gene is activated transcriptionally during the 5th instar. It is of interest to elucidate how the two genes, located on different chromosomes, are expressed coordinately. It has been shown that the 5'-flanking region of the sericin gene, which is expressed in the middle silk gland, contains a homologous sequence to the one in the 5'-flanking region of the fibroin H-chain gene[30]. The L-chain cDNA clones constructed in this study should serve as useful probes in screening L-chain genomic clones from the library, which is a prerequisite for the determination of base sequences of the L-chain gene and its flanking regions.

1 Acknowledgments. We thank Dr A. Hyodo, Dr K. Tsutusmi and Mr F. Takei for their valuable advice, and Mr A. Noda, of the Research Institute, Takara Shuzo Co. Ltd, for generously providing us with RAV-2 reverse transcriptase. This work was supported by grant No. 58470097 from the Ministry of Education, Science and Culture, Japan.

2 To whom correspondence should be addressed.
3 Present address: Laboratory of Biology, Tohoku Fukushi University, Sendai, 980, Japan.

4 Shimura, K., Kikuchi, A., Ohtomo, K., Katagata, Y., and Hyodo, A., J. Biochem. *80* (1976) 639.
5 Shimura, K., Kikuchi, A., Katagata, Y., and Ohtomo, K., J. seric. Sci., Tokyo *51* (1982) 20.
6 Shimura, K., Experientia *39* (1983) 441.
7 Couble, P., Garel, A., and Prudhomme, J.C., Devl Biol. *82* (1981) 139.
8 Couble, P., Moine, A., Garel, A., and Prudhomme, J.C., Devl Biol. *97* (1983) 398.
9 Suzuki, Y., and Brown, D.D., J. molec. Biol. *63* (1972) 409.
10 Ohmachi, T., Nagayama, H., and Shimura, K., FEBS Lett. *146* (1982) 385.
11 Hyodo, A., Gamo, T., and Shimura, K., Jap. J. Genet. *55* (1980) 297.
12 Hyodo, A., Yamamoto, T., Ueda, H., Takei, F., Kimura, K., and Shimura, K., Jap. J. Genet. *59* (1984) 285.
13 Ohshima, Y., and Suzuki, Y., Proc. natn. Acad. Sci. USA *74* (1977) 5363.
14 Tsujimoto, Y., and Suzuki, Y., Cell *18* (1979) 591.
15 Greene, R.A., Morgan, M., Shatkin, A.J., and Gage, L.P., J. biol. Chem. *250* (1975) 5114.
16 Aviv, H., and Leder, P., Proc. natn. Acad. Sci. USA *69* (1972) 1408.
17 Lizardi, P.M., and Engelberg, A., Analyt. Biochem. *98* (1979) 116.
18 McMaster, G.K., and Carmichael, G.G., Proc. natn. Acad. Sci. USA *74* (1977) 4835.
19 Laemmli, U.K., Nature *227* (1970) 680.
20 Chamberlain, J.P., Analyt. Biochem. *98* (1979) 132.
21 Oyama, F., Mizuno, S., and Shimura, K., J. Biochem., Tokyo *96* (1984) 1689.
22 Norgard, M.V., Tocci, M.J., and Monahan, J.J., J. biol. Chem. *255* (1980) 7665.
23 Norgard, M.V., Keem, K., and Monahan, J.J., Gene *3* (1978) 279.
24 Grunstein, M., and Hogness, D.S., Proc. natn. Acad. Sci. USA *72* (1975) 3961.
25 Marko, M.A., Chipperfield, R., and Birnboim, H.C., Analyt. Biochem. *121* (1982) 382.
26 Parnes, J.R., Velan, B., Felsenfeld, A., Ramanathan, L., Ferrini, U., Appella, E., and Seidman, J.G., Proc. natn. Acad. Sci. USA *78* (1981) 2253.
27 Sprague, K.U., and Roth, M.B., Cell *17* (1979) 407.
28 Thomas, P.S., Proc. natn. Acad. Sci. USA *77* (1980) 5201.
29 Ueda, H., Hyodo, A., Takei, F., Sasaki, H., Ohshima, Y., and Shimura, K., Gene *28* (1984) 241.
30 Okamoto, H., Ishikawa, E., and Suzuki, Y., J. biol. Chem. *257* (1982) 15192.

0014-4754/85/091167-05$1.50 + 0.20/0

Differences in cardiac myosin light chain LC1 among human, monkey and sheep

Yew Phew See, U. Danilczyk, P.M. Olley and G. Jackowski[1]

Cardiovascular Research Focus, Division of Cardiology, Research Institute, The Hospital for Sick Children, Toronto (Ontario, Canada M5G 1X8), 18 October 1984

Summary. The atrial and ventricular myosin light chains of human, monkey and sheep hearts were compared by dodecylsulfate polyacrylamide gel electrophoresis. The atrial light chain 2 and ventricular light chain 2 are similar among these mammals. However, the atrial light chain 1 of monkey has different electrophoretic mobility from those of human and sheep. The monkey ventricular light chain 1 has same mobility as that of sheep but different from that of human.
Key words. Monkey cardiac myosin; human cardiac myosin; sheep cardiac myosin; atrial myosin light chains; ventricular myosin light chains.

Myosin is the major contractile protein of the myofibril. The myosin molecule is a large ($M_r \sim 500{,}000$), asymmetric protein consisting of a rod-like tail and two globular heads[2,3]. It is comprised of two heavy chains ($M_r \sim 200{,}000$) and two pairs of light chains. The heavy chains interact with each other to form both the rod-like tail and the globular heads. The light chains are located in close proximity at two different sites at the globular head regions. The light chain compositions of cardiac myosins are heterogenous. The atrial and ventricular myosin light chains of mammalian hearts can be separated by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) into four different polypeptides; atrial light chain 1 (ALC1), atrial light chain 2 (ALC2), ventricular light chain 1 (VLC1) and ventricular light chain 2 (VLC2)[4-10]. The order of migration in SDS polyacrylamide gel (with increasing electrophoretic mobility) for lower mammals are, in general: VLC1, ALC1, ALC2 and VLC2[5-7]. In the case of human cardiac myosin, ALC1 migrates slower than VLC1[7-10]. Because of this difference between humans and the lower mammals and our interest in studying human cardiac myosin light chains in relation to human heart diseases[11], we have compared the light chains of human and monkey. Monkey, being a primate, may have similar cardiac myosin light chain to that of the human. In this report, we present results on the differences in electrophoretic mobility of ALC1 and VLC1 among human, monkey and sheep.

Materials and methods. Mature sheep hearts were obtained from a local abattoir. Adult monkey (*Cercopithecus Aethiops*, African Green) hearts were purchased from Connaught Laboratory, Ltd, Toronto, Canada. Human heart tissues were obtained immediately after death from a 22-year-old male accident victim with unknown medical history. All tissues were stored in liquid nitrogen until used. Myosins were prepared as described by Wikman-Coffelt et al.[12]. Protein was determined by the procedure of Bradford[13] using rabbit γ-globulin as standard. SDS

polyacrylamide gel were as described by Laemmli[14]. Proteins on gels were stained either with coomassie-blue[15] or by the silver staining procedure[16].

Results and discussion. The myosin preparation from atrial and ventricules of human, monkey and sheep were separated by SDS-PAGE (fig. 1). Myosin light chains (M_r between 20,000 and 30,000)[4-10] are the major low mol.wt components of the purified myosins. In the monkey myosin preparation (lanes 5 and 6) a polypeptide with M_r of about 27,000 is also present, and may represent contaminating troponin I which has M_r of 27,000[17]. In the human atrial preparation (fig. 1, lane 7), three bands are observed. Apart from the two higher bands, which are atrial light chains ALC1 and ALC2[7-10], a polypeptide with the same mobility as the ventricular light chain VLC2 is also present. The presence of VLC2 in the right atrial myosin preparations had previously been shown in human cardiac tissues obtained from patients with tricuspid value disease or with cardiomyopathy of unknown origin[18] and in atrial tissues of children with membranous ventricular septal defects[11]. Its presence in this specimen is unexplained.

The left and right atrial myosin preparations from sheep show the same light chain compositions (fig. 1, lanes 2 and 3), which accords with published data for other mammals, including the human[6,8,10]. The ALC2 of sheep, monkey and human (lanes 2, 3, 5 and 7) have similar electrophoretic mobilities. This is also the case for the VLC2 (lanes 4, 6, and 8).

In contrast, the mobilities of both ALC1 and VLC1 among the three mammals vary. Using the sheep, VLC1 (lane 4), as a reference, the sheep ALC1 (lanes 2 and 3) migrated significantly faster, whereas the human ALC1 (lane 7) migrated slower. The monkey ALC1 and VLC1 (lanes 5 and 6) have similar mobility as sheep VLC1. The human VLC1 migrated significantly faster than sheep VLC1 (lane 8). These differences observed for ALC1 and VLC1 from the different animal species cannot be due to the differences in protein interactions or in sample preparations. Mixing human and sheep atrial myosin samples reproduces their relative mobilities on SDS-PAGE (fig. 2).

The isoelectric point of monkey ALC1 is slightly more acidic than that for VLC1 (fig. 3). This result is similar to those obtained for rat[19,20], bovine[21] and human[9,22]; but different from those found for rabbit[7], sheep[23] and birds[20,24] where ALC1 and VLC1 have the same isoelectric point. Although monkey ALC1 and VLC1 have the same mol.wt (fig. 1), a slight separation is observed in the SDS-dimension of the 2-dimension (2D) gel (fig. 3). The length of the 2D gel, in the SDS-gel direction, is only 6.5 cm as compared to 12.5 cm for that shown in figure 1. A possible explanation for the separation of ALC1 and VLC1 in the shorter gel may be the presence of urea and ampholyte in the

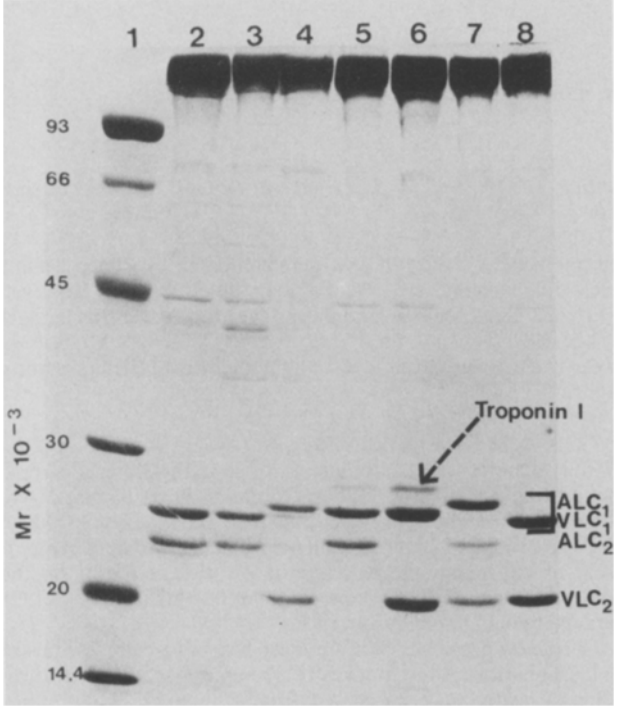

Figure 1. Sodium dodecysulfate polyacrylamide gel electrophoresis of cardiac myosin from sheep, monkey and human. 80 µg of purified myosin was separated on 10% gel and the gel was stained with coomassie-blue. *1* Mol.wt standards; *2* sheep, right atrial; *3* sheep, left atrial; *4* sheep, left ventricle; *5* monkey, left atrial; *6* monkey, left ventricle; *7* human, right atrial; *8* human, left ventricle.

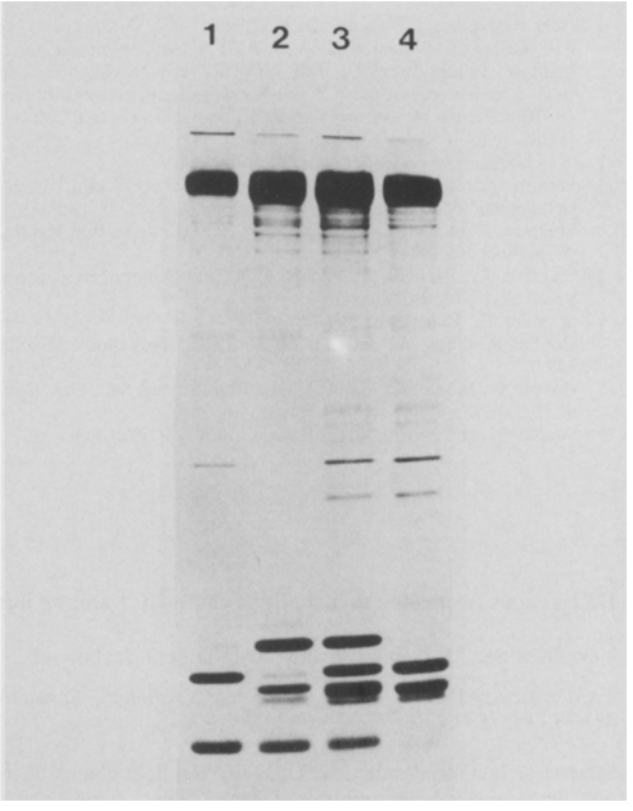

Figure 2. Co-electrophoresis of human and sheep atrial myosin. Electrophoresis was on a 15% SDS-polyacrylamide gel and protein was stained with silver. *1* Human, left ventricle (16 µg); *2* human, right atrial (16 µg); *3* mixture of human, right atrial (10 µg) and sheep, right atrial (10 µg); *4* sheep, right atrial (16 µg).

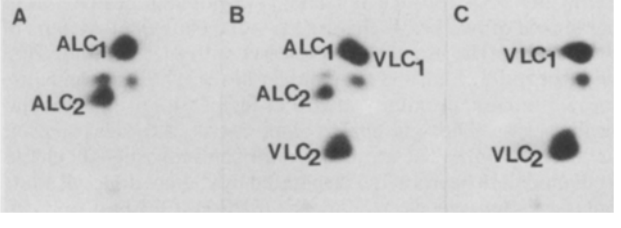

Figure 3. Two-dimensional electrophoresis of monkey atrial and ventricular myosin light chains. Electrophoresis, isoelectric focussing and silver staining were as described previously[23]. *A* Left atrial myosin (16 µg); *B* left atrial and left ventricular myosins (10 µg each); *C* left ventricular myosin (16 µg). The acidic pH range is to the left and the basic range to the right.

isoelectric focussing gel which altered the subsequent interaction between the polypeptides and SDS. The formation of doublets in ALC2 and VLC2 (fig. 3) which we observed consistently in our 2D gel system[23] may be similarly explained. Monkey VLC2 has slightly more acidic isoelectric point than ALC2 and this is in accordance with published data for other mammals and birds[7,9,19-24].

We have shown here that although human and African Green monkey are both primate, they have different light chain 1 for atrial and ventricular myosins. Our results indicate that this species of monkey would be no better than lower mammals for the study of myosin light chains in relation to human heart diseases.

1 Acknowledgments. This work was supported by MRC of Canada No. MA-8559. Dr G. Jackowski is a scholar of the Medical Research Council of Canada, and is the author to whom all correspondence should be addressed.

2 Matsuda, G., Adv. Biophys. *16* (1983) 185.
3 Harrington, W. F., and Rodgers, M. E., A. Rev. Biochem. *53* (1984) 35.
4 Lowey, S., and Ribsby, M. E., Nature *234* (1971) 81.
5 Long, L., Fabian, F., Mason, D. T., and Wikman-Coffelt, J., Biochem. biophys. Res. Commun. *76* (1977) 626.
6 Syrovy, I., Delcarye, C., and Swynghedauw, B., J. molec. cell. Cardiol. *11* (1979) 1129.
7 Srihari, T., Tuchschmid, C. R., Hirzel, H. O., and Schaub, M. C., Comp. Biochem. Physiol. *72B* (1982) 353.
8 Price, K. M., Littler, W. A., and Cummings, P., Biochem. J. *191* (1980) 571.
9 Cummins, P., Biochem. J. *205* (1982) 195.
10 Grandier-Vazeille, X., Tetaert, D., Hemon, B., and Biserte, G., Comp. Biochem. Physiol. *76B* (1983) 263.
11 Danilczyk, U., Williams, W. G., Trusler, G., See, Y. P., Olley, P., and Jackowski, G., Abstract, International Society of Heart Research, Oklahoma City, 13–15 September 1984: J. molec. cell. Cardiol. (1984) in press.
12 Wikman-Coffelt, J., Zelis, R., Fenner, C., and Mason, T., Preparative Biochem. *3* (1973) 439.
13 Bradford, M., Analyt. Biochem. *72* (1976) 248.
14 Laemmli, U. K., Nature *227* (1970) 680.
15 Jackowski, G., and Liew, C. C., Analyt. Biochem. *102* (1980) 321.
16 Oakley, B. R., Kirsch, D. R., and Morris, R. N., Analyt. Biochem. *105* (1980) 361.
17 Brekke, C., and Grease, M. L., J. biol. Chem. *251* (1976) 866.
18 Cummins, P., Perspective cardiovasc. Res. *7* (1983) 417.
19 Srihari, T., Tuchschmid, C. R., and Schaub, M. C., Basic Res. Cardiol. *77* (1982) 599.
20 Dalla Libera, L., Carraro, U., and Pauletto, P., Basic Res. Cardiol. *78* (1983) 671.
21 Whalen, R. G., Sell, S. M., Eriksson, A., and Thornell, L. E., Devl Biol. *91* (1982) 478.
22 Klotz, C., Dechesne, C., Cardinaud, R., and Leger, J. J., Biochimie *65* (1983) 569.
23 See, Y. P., Olley, P. M., and Jackowski, G., Basic Res. Cardiol. (in press).
24 Dalla Libera, L., Sartore, S., and Schiaffino, S., Biochim. biophys. Acta *581* (1979) 283.

0014-4754/85/091171-03$1.50 + 0.20/0

Dendritic reticulum cells in AIDS-related lymphadenopathy

M. Alavaikko[1], A. Rinne, M. Järvinen, V. K. Hopsu-Havu, P. R. Meyer, A. M. Levine and R. J. Lukes

Department of Pathology, University of Oulu, Oulu (Finland), Department of Dermatology, University of Turku, Turku (Finland), and Department of Pathology and Department of Medicine, University of Southern California, Los Angeles (California 90033, USA), 20 March 1985

Summary. One of two cases of acquired immune deficiency syndrome-related persistent generalized lymphadenopathy revealed a profoundly altered pattern of dendritic reticulum cells as demonstrated by immunoreactive acid cysteine proteinase inhibitor. The alterations could be related to totally or partially destructed lymphoid secondary follicles.
Key words. Immunohistochemistry; immunologic deficiency syndromes; lymph nodes; protease inhibitors.

Ultrastructural alterations, including both hypertrophy and degeneration of follicular dendritic cells or dendritic reticulum cells (DRC), have been reported in patients with acquired immunodeficiency syndrome(AIDS)-related persistent generalized lymphadenopathy (PGL)[2,3]. Recently, it has been shown that the so-called acid cysteine proteinase inhibitor (ACPI)[4] is a common characteristic of human squamous epithelia and DRC of lymphoid secondary follicles and can be demonstrated immunohistochemically in DRC in paraffin-embedded tissues[5,6]. We have analyzed two PGL cases in order to detect whether any alterations occur in ACPI-immunoreactive DRC in this disorder. The PGL cases came from the metropolitan area of Los Angeles and fulfilled the criteria of the Centers for Disease Control for PGL[7]. Lymph nodes had been fixed in B5-fixative and embedded in paraffin for routine evaluation. For demonstration of ACPI-immunoreactive DRC in the histological section, the peroxidase-antiperoxidase method after Sternberger et al.[8] was used with slight modification. In the control cases (nonspecific follicular reactive hyperplasia) a typical dendritic pattern was found consistently in the lymphoid secondary follicles (figs 1 and 2). PGL case No. 1: In hematoxylin-eosin (HE) stained sections a follicular hyperplasia was encountered without any clearcut destruction of the secondary follicles. The immunostaining for ACPI-reactive DRC exhibited a follicular pattern comparable with that of the control cases. PGL case No. 2: HE-stained sections revealed partial, subtotal or total destruction of the lymphoid secondary follicles. An ACPI-positive follicular DRC-pattern comparable with that of the control cases was found in part of the lymph node and could be related to areas where preserved secondary follicles were discernible. In areas where partial or total destruction of the secondary follicles had occurred, however, a peculiar pattern of ACPI-immunoreactive reticulum cells could be demonstrated (figs 3 and 4). The main characteristics of these areas were as follows: 1) a loss of the normal follicular DRC-pattern, 2) haphazardly organized DRC which tightly embraced groups of lymphoid cells and, 3) occasional hypertrophy of DRC. Some areas exhibited an intermediate pattern with partially preserved or destroyed follicular DRC pattern.

Out of about 100 lymph nodes representing various types of reactive lymphadenopathies, Hodgkin's disease and non-Hodgkin's lymphoma, our case No. 2 of PGL is the first where this type of ACPI-immunoreactive pattern has been encountered. The question of how consistently this phenomenon is associated