5 Benya, P. D., and Brown, P. D., Articular Cartilage Biochemistry. Raven Press, New York 1986.
6 Solavagione, E., Bourbouze, R., Percheron, F., Hecquet, C., and Adolphe, M., Biochimie *69* (1987) 239.
7 Larno, S., Bourbouze, R., Adolphe, M., Lechat, P., and Percheron, F., Cell. molec. Biol. *35* (1989) 173.
8 Lefebvre, V., Peeters-Joris, C., and Vaes, G., Biochim. biophys. Acta *1051* (1990) 266.
9 Kimura, T., Yasui, N., Ohsawa, S., and Ono, K., Clin. Orthop. *186* (1984) 231.
10 Dewilde, B., Benel, L., Hartmann, D. J., and Adolphe, M., Cytotechnology *1* (1988) 123.
11 Green, W. T., Clin. Orthop. *75* (1971) 248.
12 Ronot, X., Hecquet, C., Jaffray, P., Guiguet, M., Adolphe, M., Fontagne, J., and Lechat, P., Cell Tissue Kinet. *16* (1983) 531.
13 Dominice, J., Levasseur, C., Larno, S., Ronot, X., and Adolphe, M., Mech. Ageing Dev. *37* (1986) 231.
14 Sarber, R., Hull, B., Merill, C., Soranno, T., and Bell, E., Mech. Ageing Dev. *17* (1981) 107.
15 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
16 Ronot, X., Gaillard-Froger, B., Hainque, B., and Adolphe, M., Cytometry *9* (1988) 436.
17 Väänänen, K., Morris, D. C., Munoz, P. A., and Parvinen, E. K., Acta Histochem. *82* (1987) 211.
18 Kato, Y., and Iwamoto, M., J. biol. Chem. *265* (1990) 5903.
19 Dronne, N., Benel, L., Thenet, S., Larno, S., Mokonjimobe, E., Bourbouze, R., and Adolphe, M., Cytotechnology *2* (1989) 233.

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Evidence for the extranuclear localization of thymosins in thymus

O. E. Tsitsiloni[a], P. P. Yialouris[a], H. Echner, W. Voelter and A. A. Haritos[a]*

[a] *Zoological Laboratory, Department of Biology, University of Athens, Panepistimiopolis, GR 157 84 Athens (Greece), and Abteilung für Physikalische Biochemie, Physiologisch-chemisches Institut der Universität Tübingen, Hoppe-Seyler Str. 4, D-7400 Tübingen (Germany)*
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Abstract. A new radioimmunoassay has been developed for thymosin β_4 by generating rabbit polyclonal antibodies against the synthetic N-terminal peptide fragment 1–15 coupled to KLH. The synthetic analogue [Tyr^{12}]-thymosin β_4 (1–15) was used as tracer. This radioimmunoassay, with a useful range of 10–1000 pmoles, showed cross-reactivity with the second homologous β-thymosin of man and rat (thymosin β_{10}) but not of calf (thymosin β_9). This radioimmunoassay, together with an improved radioimmunoassay for the N-terminus of parathymosin α, was employed for the measurement of the levels of thymosin β_4 and parathymosin α in nuclear and extranuclear extracts of calf thymus. The bulk of these polypeptides was found in the extranuclear material whereas only traces were observed in the nuclear environment, which indicates the extranuclear localisation of α- and β-thymosins.
Key words. Thymosin β_4; parathymosin α; thymosin; thymus.

Thymosins, polypeptides initially isolated from thymus, belong to α- and β-homologous families. The α-family contains prothymosin α^1 and parathymosin α^2, which have approximately 100 residues. The β-family contains thymosin β_4[3] and thymosin β_9, in calf[4], or thymosin β_{10} in human[5] and rat[6] tissues, with approximately 40 residues. Thymosins are widely distributed in mammalian tissues[7,8], including blood plasma[9,10]. They can be identified by chromatographic analysis and specific radioimmunoassays[11–16]. Despite the wide distribution of thymosins, little is known about their physiological role. Reports have indicated the involvement of thymosins in cell-mediated immunity phenomena[17–20] and cell proliferation[21–24].

Conflicting results exist as to the intracellular location of prothymosin α, suggesting its presence inside the nucleus[25,26], outside the nucleus[27–30] and in both compartments[31]. We present here results showing the extranuclear location of two other thymosins, i.e. thymosin β_4 and parathymosin α, by cellular fractionation and measurement of the cross-reactive materials by radioimmunoassay.

Materials and methods

Materials. Thymus was collected from a 14-month-old calf. Fractionation of nuclear and extranuclear material and whole tissue extraction was carried out as previously reported[30]. Bovine lung thymosin β_4 and thymosin β_9[32], rat thymus thymosin β_{10}[6], human thymus prothymosin α[33] and rat liver parathymosin α[17] were isolated by established procedures.

Radioimmunoassay for thymosin β_4. Peptide fragment thymosin β_4 (1–15) and the analog [Tyr^{12}]-thymosin β_4 (1–15) were prepared by solid phase synthesis on an automatic Synostat-peptide synthesizer (Biotronik, Maintal, Germany) using 4-(hydroxymethyl)-phenoxymethyl-copoly-(styrene-1% divinyl-benzene)-resin preloaded with FMOC-Ser (tBu)-OH (for each synthesis 0.33 g = 0.26 mmol). Both syntheses were performed by the BOP/HOBt coupling procedure using FMOC amino acids with tert.-butyl side chain protecting groups. All amino acids were coupled twice in DMF in threefold excess in the presence of 0.35 g BOP/0.11 g HOBt and 1.6 ml diisopropylethylamine (25% in DMF). For the synthesis of [Tyr^{12}]-thymosin β_4 (1–15) FMOC-Phe-OH

was replaced by FMOC-tyr (tBU)-OH. After completion of the synthesis peptides were cleaved from the resins after acetylation with acetic anhydride/pyridine in dichloromethane (2 h, 0.48 ml Ac_2O/0.4 ml pyridine), using a mixture of trifluoroacetic acid/thioanisole/ethanedithiol (20:5:1; 26 ml, 3 h at room temperature). After the resins had been filtered off, the peptides were precipitated with absolute diethyl ether and washed thoroughly with dichloromethane and diethyl ether. The crude peptides were purified by liquid chromatography on a TSK-HW-40 (S) column (1.6 × 90 cm, elution with 10% acetic acid), and semipreparative HPLC using the Biotronik System (pumps BT 8100, UV-detector BT 3030) and a Nucleosil 7 C_{18} column (250 × 10 mm; gradient elution: 20 → 60% B in 30 min (A: 0.05% TFA, B: 60% acetonitrile in 0.05% TFA). The identity of the synthetic peptides was proved by amino acid analysis and mass spectroscopy.

For coupling to keyhole limpet hemocyanin (KLH), 6 mg of synthetic thymosin β_4 (1–15) and 6 mg of KLH were dissolved in 5 ml of phosphate buffered saline. Coupling was carried out with 120 µl of glutaraldehyde added in 20-µl aliquots with stirring. Stirring continued for 3 h for the completion of the reaction and the sample was dialyzed against 1 l of phosphate buffered saline overnight at 4 °C.

For each immunization an aliquot corresponding to 0.5 mg of thymosin β_4 (1–15) was mixed with an equal volume of complete Freund's adjuvant for the preparation of an emulsion, which was given to rabbits by multiple intradermal injections. Antisera were collected 1 week after the third and the fourth immunizations carried out at monthly intervals. Two out of three rabbits gave adequate titers. The antiserum used was designated L1.

The synthetic analog [Tyr^{12}]-thymosin β_4 (1–15) was radioiodinated by the chloramine T method as previously reported for tracers prepared for α-thymosin radioimmunoassays[15,16]. A quantity of 2.5 mCi of ^{125}I (Amersham) was added to 1.5 µg of the synthetic peptide. The radioiodinated peptide was purified from excess $Na^{125}I$ by forcing the sample through a Sep-Pak C18 cartridge equilibrated with 1 M HCOOH/0.2 M pyridine (buffer A). The cartridge was washed with 20 ml of buffer A and the bound peptide was eluted with 20% propanol solution in buffer A.

For the radioimmunoassay, 12 µl of antiserum, 13 µl of preimmune serum, 100 µl of tracer solution corresponding to 30,000 cpm and 0.2 M phosphate buffer pH 7.0 to a final volume of 500 µl, were mixed in 5.0-ml plastic tubes. Samples were incubated overnight at 4 °C. The immune complexes were precipitated by the addition of 500 µl of saturated ammonium sulphate. 30 min later the tubes were centrifuged for 10 min at 5,000 rpm in a Heraeus Megafuge centrifuge. The supernatants were removed by aspiration and the pellets counted in a LKB γ-counter, model Minigamma.

Radioimmunoassay for parathymosin α. A radioimmunoassay previously reported for parathymosin α[16] has been improved in sensitivity by generating antibodies against the N-terminal synthetic peptide parathymosin α (1–30), corresponding to the corrected N-terminal sequence of parathymosin α[34]. The synthetic analog [Tyr^{11}]-parathymosin α (1–10) was used for labeling. These synthetic peptides were generously provided by Drs E. Heimer and A. Felix of Hoffmann-La Roche Inc. (USA).

HPLC gel-filtration. Gel-filtration was performed at room temperature in a Waters Ass. HPLC system equipped with an LKB Ultropac column TSK G2000SW (7.5 × 600 mm) and a TSKSW9 precolumn. The buffer used was 0.2 M phosphate buffer pH 7.0. The sample was filtered through 0.45-µm Sartorius filters (Minisart NML SM 16555).

Results and discussion

Thymosins were given this name because they were initially isolated from thymus. Thymosin α_1, the N-terminal segment 1–28 of prothymosin α, and thymosin β_4, were the first to be discovered[3,35]. Prothymosin α^1 and parathymosin α^2[,17], partly homologous with prothymosin α, as well as thymosins β_9[4] and β_{10}[5,36], both homologous with thymosin β_4, were discovered later on. Although thymosins have since been found in almost every tissue analyzed, the thymus still remains a major source of thymosins; it is the richest source for prothymosin α and the second richest for thymosin β_4 (after spleen)[7,37].

It was proposed that prothymosin α is a nuclear polypeptide, on the basis of its structure, as it contains segments of dicarboxylic acid residues, and a putative signal peptide for accumulation in the nucleus (B/Thr Lys Lys Z Lys, where B stands for basic residues and Z for a polar residue)[38]. However, experimental data are conflicting. Radiolabeled prothymosin α injected into the cytoplasm of *Xenopus* oocytes was found to be transfered to the nucleus[25]. Also, constructs in which human prothymosin α nucleic acid sequence was fused in-frame near the amino terminus of a β-galactosidase gene expressed in COS cells resulted in nuclear localization of the fusion protein[26]. However, experiments using indirect immunofluorescence and immunoelectron microscopy with monoclonal and polyclonal antibodies against thymosin α_1 showed the accumulation of cross-reactive material in the cytoplasm[27–29], especially in cytoplasmic vacuoles of thymic reticuloepithelial cells[28,29]. Measurements of the cross-reactive material in isolated nuclei and extranuclear material in lymphoid and non-lymphoid calf tissues also showed an extranuclear localization[30]. The finding of low molecular-weight cytoplasmic RNA covalently bound to prothymosin α is in agreement with an extranuclear location for prothymosin α[39,40]. Recently, immunoelectron microscopy showed the presence of thymosin α_1 cross-reactive material both inside and outside the

nucleus (although it was claimed that the staining density was higher inside the nucleus) in cells of a rat small-intestinal cell line[31].

In the present study, we extended the studies of intracellular distribution to two other thymosins, parathymosin α and thymosin β_4. For this purpose, radioimmunoassays were developed for the measurement of these thymosins in isolated nuclear and extranuclear samples in calf thymus.

The radioimmunoassay for thymosin β_4 employs an antiserum generated against the synthetic thymosin β_4 fragment 1–15 coupled to KLH by glutaraldehyde, and the radiolabeled synthetic analog [Tyr[12]]-thymosin β_4 (1–15). It was found to cross-react with the intact thymosin β_4 polypeptide (fig. 1). The useful range of this radioimmunoassay is 10–1000 pmoles of thymosin β_4 per tube. Previous radioimmunoassays for thymosin β_4[12–13] were based on antisera generated against isolated intact thymosin β_4, with tracers being either tritiated thymosin β_4[12] or (^{125}I)[Tyr[1]]-thymosin β_4 (31–43)[13]. Their useful range was 5–100 pmoles and 0.1–10 pmoles of thymosin β_4, respectively. Although the present radioimmunoassay is less sensitive, it represents the first radioimmunoassay for thymosin β_4 based on antiserum generated against a synthetic fragment of thymosin β_4 and thus specific for the N-terminus of this polypeptide.

The antiserum of the present radioimmunoassay does not cross-react with the second β-thymosin in calf tissues, i.e. thymosin β_9, or the structurally unrelated α-thymosin, prothymosin α (fig. 1). However, it does cross-react with thymosin β_{10} (present as the second β-thymosin in rat and human tissues) (fig. 1). The primary structure of thymosin β_4 differs from those of thymosins β_9 and β_{10} at position 1 (Ser, Ala, Ala), 7 (Ala, Gly, Gly), 10 (Glu, Asn, Ala), 11 (Lys, Ser, Ser) and 15 (Ser, Ala, Ala). However, in position 6 thymosin β_4 differs only from thymosin β_9 (Met, Leu) but not from thymosin β_{10} (Met, Met). It is therefore possible that this is why this antiserum cross-reacts significantly with thymosin β_{10} but not with thymosin β_9. This speculation is corroborated by the report that an antiserum against intact thymosin β_4 recognized two major epitopes located in sequences 1–8 and 22–32[12], the former containing the methionine residue at position 6. A similar cross-reactivity of 21% has previously been reported for an antiserum against intact thymosin β_4 with thymosin β_{10}[8]. Our finding of a lack of cross-reactivity of the antiserum against thymosin β_4 with thymosin β_9 appears to be valid in the opposite direction as well, i.e. an antiserum against the thymosin β_9 fragment 1–14 was reported not to show any cross-reactivity with thymosin β_4[41]. Therefore, with the present radioimmunoassay only thymosin β_4 was measured in calf thymus extracts, since thymosin β_9 does not cross-react, and thymosin β_{10} is not present in calf tissues.

Radioimmunoassays for parathymosin α have been developed by generating antibodies against intact parathymosin α[14] and the synthetic N-terminal fragment 1–30 of parathymosin α[16]. Tritiated parathymosin α and the radioiodinated analogue [Tyr[13]]-parathymosin α (1–12), respectively, were used as tracers. The latter radioimmunoassay, specific for the N-terminus of parathymosin α, cross-reacted with intact rat parathymosin α with a useful range of 1–20 pmoles but not with human prothymosin α and rat thymosin β_4 up to 500 pmoles (detailed results to be presented elsewhere).

The bulk of the immunologically cross-reactive material for both thymosins was found in the extranuclear fraction, with traces only being observed in the nuclear fraction (fig. 2). For parathymosin α, the cross-reactive material extracted from the isolated thymic nuclei was 6.5%

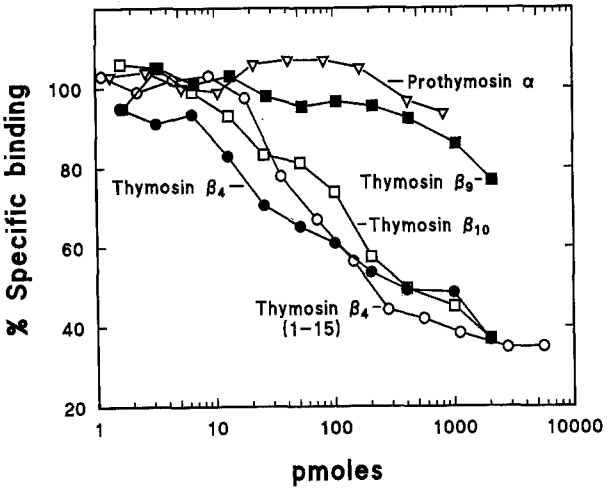

Figure 1. Comparison of the radioimmunoassays for thymosin β_4, its fragment thymosin β_4 (1–15), its homologous polypeptides thymosin β_9 and thymosin β_{10}, and the α-thymosin prothymosin α. These compounds were separately incubated with the tracer [Tyr[12]]-thymosin β_4 (1–15) over the indicated concentration range. Subsequent displacement of the tracer was assessed and any cross-reactivity indicated by calculating the percentage of tracer remaining bound to the antiserum.

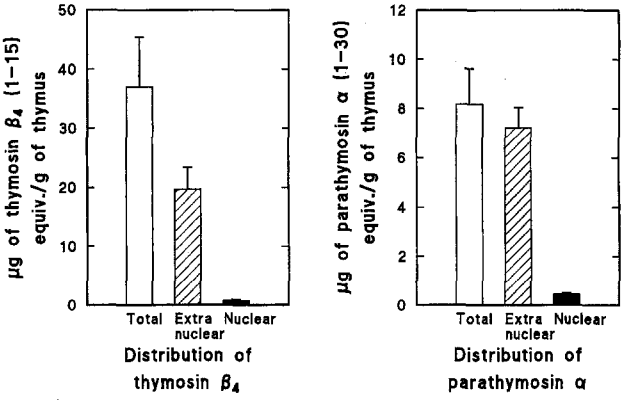

Figure 2. Levels of thymosin β_4 and parathymosin α cross-reactive materials in cellular fractions of calf thymus. Total, whole tissue extract. Aliquots of total, extra-nuclear and nuclear fractions of 100, 150 and 500 μl for the thymosin β_4 radioimmunoassay and 50, 70 and 400 μl for the parathymosin α radioimmunoassay respectively, were analyzed.

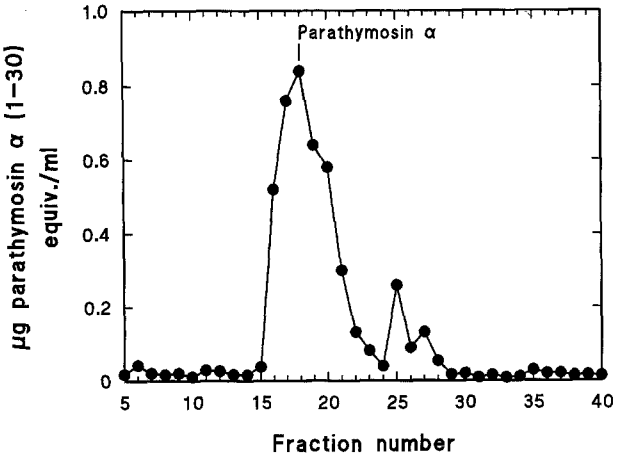

Figure 3. Separation by HPLC gel-filtration of peptides in the extranuclear material. A volume of 500 μl corresponding to 1 g of fresh thymus was injected. The flow rate was 0.5 ml/min and 1-ml fractions were collected. Aliquots of 70 μl of the collected fractions were directly analyzed by the parathymosin α radioimmunoassay. The perpendicular arrow indicates the position corresponding to the elution volume of parathymosin α.

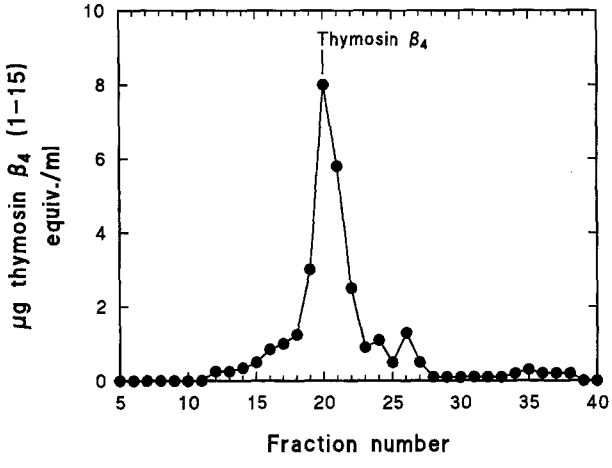

Figure 4. Separation by HPLC gel-filtration of peptides in the extranuclear material. Aliquots of 100 μl from the collected fractions were directly analyzed by the thymosin β_4 radioimmunoassay. The perpendicular arrow indicates the position corresponding to the elution volume of thymosin β_4. Other details as in figure 3.

of that in the extranuclear compartment. The equivalent value for thymosin β_4 was 4.0%. The combined intra- and extra-nuclear material cross-reacting with parathymosin α accounted for 94% of the total cross-reactive material extracted by extensive homogenization of whole tissue. In the case of thymosin β_4, the value was only 55%. The reason for the lower value is not clear. The cross-reactive materials were further identified as intact parathymosin α (fig. 3) and thymosin β_4 (fig. 4) by their elution volumes in gel-filtration HPLC. The peaks of cross-reactive materials corresponded to the elution volumes of isolated parathymosin α and thymosin β_4, respectively[42,43].

It has been reported that parathymosin α inhibits the key glycolytic enzyme phosphofructokinase-1 through a Zn^{2+} dependent binding to the phosphofructokinase-1 protomers, so that a role of parathymosin α may be the regulation of cytosolic enzyme activity(ies)[44,45]. Such a role would be consistent with an extranuclear location of this peptide. Using immunocytochemistry, parathymosin α was observed outside the nucleus in many tissues[46]. In rat brain, parotid glands, intestine and pancreas cells it was localized in the cytoplasm. In duodenal and jejunal crypt cells, however, immunostaining was restricted to the nucleus, whereas in more mature cells at the top of the villi parathymosin α was cytosolic[46]. In *Xenopus* oocytes injected with radiolabeled parathymosin α and thymosin β_4, the former polypeptide was found to migrate to the nucleus while the latter remained in the cytoplasm[47].

In conclusion, evidence from this and previous investigations points towards the presence of α- and β-thymosins outside the nucleus in the thymus. However, there is a strong possibility that both prothymosin α and parathymosin α are differentially distributed in the nuclear and extranuclear cellular compartments in different tissues, or at different developmental or proliferation stages. Although an extranuclear localization for thymosin β_4 has been reported[47], and present results support this, the appearance of this polypeptide in the nucleus under the conditions mentioned above for α-thymosins cannot be ruled out. Further investigation to identify the factors which may influence the intracellular localization of thymosins is needed.

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* To whom all correspondence should be addressed.

1 Haritos, A. A., Blacher, R., Stein, S., Caldarella, J., and Horecker, B. L., Proc. natl Acad. Sci. USA *82* (1985) 343.
2 Komiyama, T., Pan, L.-X., Haritos, A. A., Wideman, J. W., Pan, Y.-C. E., Chang, M., Rogers, I., and Horecker, B. L., Proc. natl Acad. Sci. USA *83* (1986) 1242.
3 Low, T. L. K., and Goldstein, A. L., J. biol. Chem. *257* (1982) 1000.
4 Hannappel, E., Davoust, S., and Horecker, B. L., Proc. natl Acad. Sci. USA *79* (1982) 1708.
5 Erickson-Viitanen, S., Ruggieri, S., Natalini, P., and Horecker, B. L., Archs Biochem. Biophys. *221* (1983) 570.
6 Haritos, A. A., Caldarella, J., and Horecker, B. L., Analyt. Biochem. *144* (1985) 436.
7 Haritos, A. A., Tsolas, O., and Horecker, B. L., Proc. natl Acad. Sci. USA *81* (1984) 1391.
8 Horecker, B. L., and Morgan, J., Lymphokines *9* (1984) 15.
9 Panneerselvam, C., Haritos, A. A., Caldarella, J., and Horecker, B. L., Proc. natl Acad. Sci. USA *84* (1987) 4465.
10 Hannappel, E., and Van Kampen, M., J. Chrom. *397* (1987) 279.
11 McClure, J. E., Lameris, W., Wara, D. W., and Goldstein, A. L., J. Immun. *128* (1981) 368.
12 Goodall, G. J., Hempstead, J. L., and Morgan, J. I., J. Immun. *131* (1983) 821.
13 Naylor, P. H., McClure, J. E., Spangelo, B. L., Low, T. L. K., and Goldstein, A. L., Immunopharmac. *7* (1984) 9.
14 Panneerselvam, C., Caldarella, J., and Horecker, B. L., J. Immun. Meth. *104* (1987) 131.
15 Yialouris, P. P., Evangelatos, G. P., Soteriadis-Vlahos, C., Heimer, E. P., Felix, A. M., Tsitsiloni, O. E., and Haritos, A. A., J. Immun. Meth. *106* (1988) 267.
16 Tsitsiloni, O. E., Yialouris, P. P., Heimer, E. P., Felix, A. M., Evangelatos, G. P., Soteriadis-Vlahos, C., Stiakakis, J., Hannappel, E., and Haritos, A. A., J. Immun. Meth. *113* (1988) 175.

17 Haritos, A. A., Salvin, S. B., Blacher, R., Stein, S., and Horecker, B. L., Proc. natl Acad. Sci. USA *82* (1985) 1050.
18 Sztein, M. B., and Goldstein, A. L., Springer Semin. Immunopath. *9* (1986) 1.
19 Salvin, S. B., Horecker, B. L., Pan, L.-X., and Robin, B. S., Clin. Immun. Immunopath. *43* (1987) 281.
20 Baxevanis, C. N., Reclos, G. J., Papamichail, M., and Tsokos, G. C., Immunopharmac. Immunotox. *9* (1987) 429.
21 Eschenfeldt, W. H., and Berger, S. L., Proc. natl Acad. Sci. USA *83* (1986) 9403.
22 Gomez-Marquez, J., Segade, F., Dosil, M., Pichel, J. G., Bustelo, X. R., and Freire, M., J. biol. Chem. *264* (1988) 8451.
23 McCreary, V., Kartha, S., Bell, G. I., and Toback, F. C., Biochem. biophys. Res. Commun. *152* (1988) 862.
24 Schobitz, B., Netzker, R., Hannappel, E., and Brand, K., J. biol. Chem. *265* (1991) 15387.
25 Watts, J. D., Cary, P. D., and Crane-Robinson, C., FEBS Lett. *245* (1989) 17.
26 Manrow, R. E., Sburlati, A. R., Hanover, J. A., and Berger, S. L., J. biol. Chem. *266* (1991) 3916.
27 Hirokawa, K., McClure, J. E., and Goldstein, A. L., Thymus *4* (1982) 19.
28 Auger, C., Stahli, C., Fabien, N., and Monier, J. C., J. Histochem. Cytochem. *35* (1987) 181.
29 Fabien, N., Auger, C., and Monier, J. C., Immunology *63* (1988) 74.
30 Tsitsiloni, O. E., Yialouris, P. P., Sekeri-Pataryas, K., and Haritos, A. A., Experientia *45* (1989) 332.
31 Conteas, C. N., Mutchnick, M. G., Palmer, K. C., Weller, F. E., Luk, G. D., Naylor, P. H., Erfos, M. R., Goldstein, A. L., Panneerselvam, C., and Horecker, B. L., Proc. natl Acad. Sci. USA *87* (1990) 3269.
32 Hannappel, E., Analyt. Biochem. *156* (1986) 390.
33 Pan L.-X., Haritos, A. A., Wideman, J., Komiyama, T., Chang, M., Stein, S., Salvin, S. B., and Horecker, B. L., Archs Biochem. Biophys. *250* (1986) 197.
34 Panneerselvam, C., Wellner, D., and Horecker, B. L., Archs Biochem. Biophys. *265* (1988) 454.
35 Low, T. L. K., and Goldstein, A. L., J. biol. Chem. *254* (1979) 987.
36 Goodall, G. J., and Horecker, B. L., Archs Biochem. Biophys. *256* (1987) 402.
37 Hannappel, E., Xu, G. J., Morgan, J., Hempstead, J., and Horecker, B. L., Proc. natl Acad. Sci. USA *79* (1982) 2172.
38 Gomez-Marquez, J., and Segade, F., FEBS Lett. *226* (1988) 217.
39 Vartapetian, A. B., Makarova, T. N., Koonin, E. V., Agol, V. I., and Bogdanov, A. A., FEBS Lett. *232* (1989) 35.
40 Makarova, T., Grebenshikov, N., Egorov, C., Vartapetian, A., and Bogdanov, A., FEBS Lett. *257* (1989) 247.
41 Mihelic, M., Kalbacher, H., Hannappel, E., and Voelter, W., J. Immun. Meth. *122* (1989) 7.
42 Haritos, A. A., Yialouris, P. P., Heimer, E. P., Felix, A. M., and Rosemeyer, M. A., FEBS Lett. *218* (1987) 107.
43 Haritos, A. A., Yialouris, P. P., Heimer, E. P., Felix, A. M., Hannappel, E., and Rosemeyer, M. A., FEBS Lett. *244* (1989) 287.
44 Brand, I. A., and Soling, H.-D., J. biol. Chem. *261* (1986) 5892.
45 Trompeter, H.-J., Brand, I. A., and Soling, H.-D., FEBS Lett. *253* (1989) 63.
46 Brand, I. A., Heinickel, A., and Soling, H.-D., Eur. J. Cell Biol. *54* (1991) 157.
47 Watts, J. D., Cary, P. D., Sautiere, P., and Crane-Robinson, C., Eur. J. Biochem. *192* (1990) 643.

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CD11b-bearing mononuclear leucocytes and IgA levels in the staging of human immunodeficiency virus infection

J. M. Rivera-Guzmán[c], J. R. Regueiro[a, b], J. Alcamí[a] and A. Arnaiz-Villena[a, d]

[a] *Inmunología, Hospital '12 de Octubre', E-28041 Madrid (Spain),* [b] *Department of Pediatry, Univ. of Valladolid,* [c] *Department of Epidemiology, Univ. Complutense of Madrid, and* [d] *Department of Medicine, Univ. of Alcalá (Spain).*
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Abstract. Certain immunological parameters (i.e. low CD4+ T cell numbers, high serum soluble CD8) have been described as prognostic factors for the progression of human immunodeficiency virus (HIV) infection to later clinical stages. In the present study we have found in one hundred HIV-infected Spanish patients (81% drug abusers, 7% homosexuals, 6% heterosexuals, and 6% other or unknown risk groups) that CD11b+ peripheral blood mononuclear cells are increased in those with persistent lymphadenopathy as compared to other clinical stages (asymptomatic, AIDS-related complex and AIDS). Serum IgA was significantly increased in AIDS patients, and in patients at any other clinical stage who had concomitant infections (mainly mycobacterial and fungal). CD11b (an integrin with complement receptor functions) may thus be of clinical interest for the staging of HIV-infected patients, and reflect stage-selective immunological changes in mononuclear cell biology during HIV infection. High IgA on the other hand, would be a marker of concomitant infection as well as of disease progression. The results concern mostly drug addicts (the main risk group in Spain), but may apply to the other risk groups because no significant differences were detected between drug addicts ($n = 81$) and non-drug addicts ($n = 19$) for the studied variables ($p > 0.05$).
Key words. Monocytes; natural immunity; IgA; CD11b; human immunodeficiency virus.

In order to understand the pathogenesis and natural history of infection with the human immunodeficiency virus (HIV) it is important to identify factors that correlate with, and possibly contribute to, the outcome of the infection. Several epidemiological studies have confirmed the crucial role of reductions in CD4+ T cells and of increases in serologic markers of lymphocyte activation (soluble CD8, CD25 and β2microglobulin) in the progression of HIV infection[1, 2]. Phenotypic and functional changes in other immune cells, like monocytes, may also be of interest, particularly since this cell type can also be a target of HIV infection, as has indeed been previously reported[3]. We therefore studied one hundred HIV-infected Spanish patients to determine the changes