Clin. exp. Immunol. (1985) **61**, 118–124.

Studies on lymphocyte cell surface in ataxia–telangiectasia

NESRIN KARTAL ÖZER,* GÖNENÇ CILIV,* A.I. BERKEL,† ÖZDEN SANAL,† O.YEĞIN† & FÜGEN ERSOY† *Department of *Biochemistry and †Paediatrics, Hacettepe University, Ankara, Turkey*

(Accepted for publication 30 January 1985)

SUMMARY

Lymphocyte surface proteins of patients with ataxia–telangiectasia were separated by polyacrylamide gel electrophoresis and compared by autoradiography. The patients lacked one of the two main bands (Band I at the origin). The second main band (Band II) was absent in some cases. All patients had one or two additional bands of smaller molecular weight than Band II except one case who had no band detectable. In the patients, alkaline phosphatase, total ATPase and Mg^{2+}. ATPase were increased but 5′-nucleotidase was normal. The results suggest abnormality in the plasma membranes of the patients' lymphocytes.

Keywords ataxia–telangiectasia lymphocyte cell surface autoradiography membrane bound enzymes

INTRODUCTION

Ataxia–telangiectasia (A–T) is an autosomal recessive disorder characterized by progressive cerebellar ataxia, oculocutaneous telangiectasia, hypersensitivity to ionizing radiation, immunodeficiency and predisposition to the development of neoplasia (Paterson & Smith, 1979). Most patients die of infections or malignancies.

Membrane abnormalities may play an important role in cellular functions of patients with immunodeficiency (Remold-O'Donnel *et al.*, 1984; Thompson, Candy & McNeish, 1984). Abnormal receptor sites in the thrombocyte membranes of an A–T patient with bleeding is reported (Arkel *et al.*, 1981). Impairment in cellular interactions between the lymphocytes in A–T patients and in patients with chronic lymphocytic leukaemia is described (Weisbart *et al.*, 1980). We studied the cell surface of A–T lymphocytes by radioiodination and by measuring the activities of some membrane bound enzymes.

MATERIALS AND METHODS

Fourteen patients (nine males and five females, aged 5–14 years) diagnosed as A–T in the Immunology Unit of the Department of Paediatrics, Hacettepe University, Ankara, Turkey were studied. Seven were examined by autoradiography. Three of these patients and seven additional cases (total of 10 cases) were included in the enzyme studies (Table 1). Fourteen controls (ages ranging between 7 and 14 years) were selected from patients admitted to the hospital for minor surgical operations; none of them had systemic disease or immunodeficiency.

Correspondence: Dr Nesrin Kartal Özer, Department of Biochemistry, Hacettepe University Ankara, Turkey.

Serum immunoglobulins (IgG, IgM, IgA) and E-rosette test were determined by described methods (Ersoy & Berkel, 1974).

To isolate lymphocytes, 10 ml venous blood drawn in heparinized syringes was mixed with 5 ml of phosphate-buffered saline (PBS; 10 mM sodium phosphate buffer containing 0·9% NaCl, pH 7·2) and layered over Ficoll-Hypaque (Sigma Chemical Co., St Louis, Missouri, USA). After centrifugation at 500 ***g***, for 30 min at 4°C the lymphocytes from the interface were collected and transferred to another tube, treated with 10 ml of 0·87% NH_4Cl to lyse erythrocytes and washed two times with PBS. They were resuspended in PBS at a concentration of 2×10^7 cells/ml (Böyum, 1968; Pettegrew, Nichols & Stewart, 1981). Less than 10% of macrophages were detected by myeloperoxidase staining and viability by trypan blue exclusion was greater than 95%.

A control was run in parallel with each case.

To iodinate and solubilize lymphocyte surface proteins, intact lymphocytes were labelled with ^{125}I by the lactoperoxidase method (Smith *et al.*, 1975). Twenty million cells were suspended in 1 ml of PBS, pH 7·4 containing 150 mM NaCl, 50 μg lactoperoxidase (Sigma; 80 u/mg), 500 mM KI and 0·5 mCi carrier free ^{125}I (Amersham, UK). The mixture was warmed to 30°C and the reaction started by the addition of 50 μl H_2O_2 (10 mM) with constant shaking. The same amount of H_2O_2 was added to the incubation mixture two more times at 5 min intervals and the reaction was terminated by the addition of 17·5 ml cold PBS. Care was taken to maintain the pH at 7·2–7·4 during incubation. The cells were washed four times in PBS and finally resuspended in 0·125 ml of the same buffer; 95% remained viable by the trypan blue dye exclusion test. Incubation tubes without lactoperoxidase and H_2O_2 constituted the blanks. The cells were shaken with equal volume of 1%(vol./vol.) Nonidet P-40 (Sigma) for 10 min at room temperature. The suspension was then centrifuged at 1,300 ***g*** for 30 min at 4°C. Protein was determined in the supernatant (Lowry *et al.*, 1951).

An aliquot of supernatant containing 60 μg protein was applied to 10 cm of 7·5% polyacrylamide gels with 0·5% SDS and electrophoresed (Weber, Pringle & Osborn, 1972). The gels were fixed in 7·5% acetic acid overnight and subjected to autoradiography directly on X-Omat films (Kodak) (Barritault & Hayes, 1973; Ho, 1979). The mol. wt of the iodinated proteins were calculated from their mobilities compared to the migration of proteins of known mol. wt (Weber *et al.*, 1972).

Enzyme activities were determined in the lymphocyte homogenates prepared in 250 mM sucrose–30 mM Tris-HCl, pH 7·4 (Dimitrov & Ellegaard, 1972), 5′-Nucleotidase, total adenosine triphosphatase (ATPase) and Mg^{2+}. ATPase were assayed by measuring the inorganic phosphate liberated in the incubation mixtures containing the lymphocyte homogenate, the appropriate substrates (5′-AMP at pH 7·4, ATP at pH 7·4 and ATP at pH 8·0, respectively), activators and inhibitors for each enzyme (Michell & Hawthorne, 1965; Dupus & Doucet, 1981; Ellegaard & Dimitrov, 1972; Dornand *et al.*, 1974; Pau, Dornand & Mani, 1976).

Inorganic phosphate (P_i) was determined by the method of Ames (1966).

Alkaline phosphatase was determined by following the liberation of *p*-nitrophenole (PNP) at pH 9·0 using *p*-nitrophenylphosphate as substrate (Neumann *et al.*, 1976; Kramers, Catavsky & Foa, 1978).

Mann–Whitney U test was used for statistical analysis (Rohlf & Sokal, 1969).

RESULTS

The only significant difference in immunological findings between the controls and patients was in the IgA levels (Table 1). IgA was not detected in six and was very low in one patient. E-rosette percentage was, also, low in five of these patients. Cases 6 and 7 were siblings.

Two bands were observed in the autoradiographs of all the controls: one band was at the origin (Band I) and the other was spread in the region corresponding to 145.000–132.000 molecular weights (Band II) (Table 2 and Fig 1).

Band I was not detected in any patient. Band II was detected in four patients. One or more additional bands of smaller molecular weights than Band II were observed in six patients (Fig 2).

Table 1. The immunological findings of patients

Case	Age (years)	Sex	Onset of ataxia (years)	Onset of telangiectasia (years)	Total lymphocytes/μl	E-rosettes (%)	Serum immunoglobulins (mg/dl)		
							IgG	IgM	IgA
1	10	F	1	4	2,921	43	1,400	253	14
2	8	M	2	4	1,408	39	1,170	129	0
3	5	F	4	2·5	1,368	ND	970	114	112
4	6	F	1	2	1,900	49	840	85	0
5*	9	M	5	3	1,548	35	1,230	175	0
6*	8	F	3	5	2,800	54	795	127	80
7*	14	M	4	3	2,100	68	1,010	115	103
8	6	M	2	6	3,136	43	1,050	121	0
9	5	M	2·5	2	3,980	51	1,170	163	75
10	9	M	2	4	ND	61	830	137	0
11	6	M	2	2	1,602	39	750	100	80
12	7	M	1	1	1,638	50	1,070	57	73
13	9	F	4	3	2,400	64	1,130	138	87
14	9	M	1·5	2	ND	ND	1,380	148	0
	Normal values				> 2,000	67 ± 7	1,200 ± 300	120 ± 35	138 ± 37

ND = not determined
Cases 1–7 were studied by autoradiography.
Cases 8–14 were studied for enzyme activities.
* Cases studied both by autoradiography and for enzyme activities.

Table 2. The radioactive labelled bands and their mol. wt

Group		Band I	Band II (mol. wt)	Additional bands (mol. wt) A	Additional bands (mol. wt) B
Control	1	+	132,000		
	2	+	145,000		
	3	+	133,000		
	4	+	135,000		
	5	+	135,000		
	6	+	133,000		
	7	+	145,000		
Patient	1	–	132,000	108,000	–
	2	–	130,000	76,000	34,000
	3	–	135,000	80,000	–
	4	–	130,000	84,000	–
	5	–	–	–	–
	6	–	–	80,000	60,000
	7	–	–	115,000	51,000

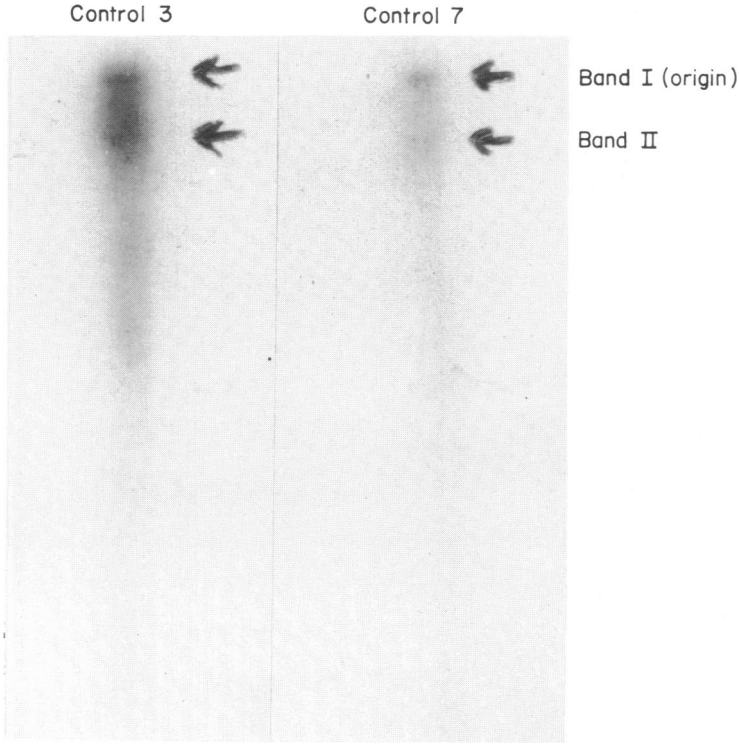

Fig. 1. Autoradiographs of two controls (arrows indicate the radioactive labelled bands)

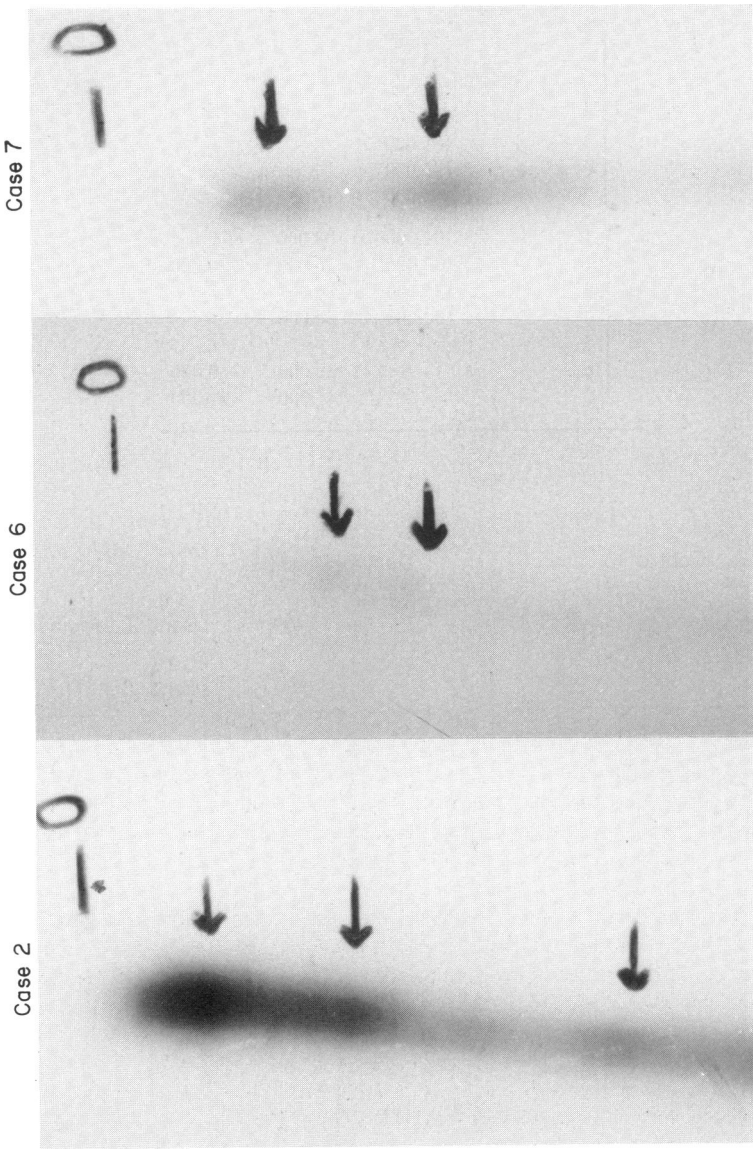

Fig. 2. Autoradiographs of three patients (arrows indicate the radioactive labelled band; 'O' stands for origin)

Table 3. The activities of some membrane bound enzymes

Group	5′-nucleotidase (nmol P_i/30 min/ mg protein) Mean ± s.d.	Alkaline phosphatase (nmol PNP/min/ mg protein) Mean ± s.d.	Total ATPase (μmol P_i/30 min/ mg protein) Mean ± s.d.	Mg^{2+}ATPase (μmol P_i/30 min/ mg protein) Mean ± s.d.
Control ($n=10$)	90·62 ± 36·22 (52·30–167·00)*	0·98 ± 0·22 (0·64–1·24)	1·45 ± 0·35 (0·97–2·15)	1·49 ± 0·43 (0·81–2·35)
Patient ($n=10$)	91·51 ± 57·11 (17·00–194·00)	1·67 ± 0·52 (1·05–2·63)	1·93 ± 0·48 (1·22–2·84)	1·94 ± 0·65 (1·13–3·06)
P	> 0·05	< 0·01	< 0·01	< 0·05

n = number of cases.
* Number of parentheses indicate ranges.

Bands I and II were not detected in the two siblings (Case 6 and 7) but they had two smaller bands of 80.000, 60.000 and 115.000, 51.000 molecular weights, respectively. Case 5 who had developed a lymphoma, revealed no radioactive labeled band. The autoradiographs prepared from lactoperoxidase, H_2O_2 free incubation mixtures lacked radioactive labeled bands.

The activities of the membrane bound enzymes, alkaline phosphatase, total ATPase and Mg^{2+}.ATPase were all increased except 5′-nucleotidase (Table 3).

DISCUSSION

In A–T patients clinical, genetic and immunological heterogeneity have been observed. Many studies in DNA synthesis and repair mechanisms, in tissue differentiation, in chromosomal translocations especially of chromosome 7 and 14, and dysfunction of the immune system have not explained this heterogeneity (Setlow, 1978; Al Saadi, Palutke & Kumar, 1980; Painter & Young, 1980; Tamura *et al.*, 1980; Murnane & Painter, 1983; Berkel *et al.*, 1984).

The autoradiographic patterns in the patient group are not consistent. The absence of Band I in all our patients and the absence of Band II and the presence of additional radioactive labelled bands in some patients show abnormality of the lymphocyte surface components in A–T patients. These may result from migration of some intrinsic proteins of smaller mol. wt to the cell surface, synthesis of abnormal cell surface proteins or conformational changes of normally synthesized cell surface proteins.

Lymphocyte surface protein abnormalities in Wiskott–Aldrich syndrome has been reported (Parkman *et al.*, 1981; Remold-O'Donnel *et al.*, 1984). Several iodination studies of lymphocyte membranes from different sources have been published (Marchalonis, Cone & Santer, 1971; Smith *et al.*, 1975; Parkman *et al.*, 1981; Williams *et al.*, 1981). The number of radioactive bands seems to vary according to the source of cells and methods used.

To our knowledge few enzymes are studied in A–T cases (Sheridan & Huang 1979). The significant increases in three membrane bound enzyme activities, also, support the autoradiographic results suggesting abnormality in A–T lymphocyte surface.

The number of cases studied both by autoradiography and for enzyme activities were limited (three cases). Therefore further studies are needed for the association of autoradiographic patterns and changes in enzyme activities.

REFERENCES

Al Saadi, A., Palutke, M. & Kumar, G.K. (1980) Evolution of chromosomal abnormalities in sequential cytogenetic studies of ataxia telangiectasia. *Hum. Genet.* **55**, 23.

AMES, B.N. (1966) Assay of inorganic phosphate, total phosphate and phosphatases. In *Methods in Enzymology* (ed. by E.F. Neufeld & V. Ginsburg) p. 115. Academic Press, New York.

ARKEL, Y.S., KINCHELOW, T.E., OLESKE, J., SEARLE, B.M. & DESPOSITO, F. (1981) Ataxia telangiectasia with thrombasthenia, platelet dysfunction, and a chromosomal translocation. A monoclonal defect? *Am. J. Pediatr. Hematol. Oncol.* **3,** 27.

BARRITAULT, D.S. & HAYES, D.H. (1973) Rapid scanning of ^{32}P-labeled acrylamide gels. *Anal. Biochem.* **53,** 272.

BERKEL, A.I., ERSOY, F., SANAL, Ö, CILIV, G. & YEĞIN, O. (1984) Immune dysfunctions in Ataxia–Telangiectasia. In *Recent Advances in Immunology* (ed. by A.Ü. Müftüoğlu & N. Barlas) p. 173. Plenum Press, New York.

BÖYUM, A. (1968) Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1*g*. *Scand. J. lab. Invest.* **21 (suppl. 27),** 77.

DIMITROV, N.V. & ELLEGAARD, J. (1972) Elevated lymphocyte adenosine triphosphate activity in patients with gastrointestinal carcinoma. *N. Engl. J. Med.* **286,** 353.

DORNAND, J., MANI, J.C., MOUSSERON-CANET, M. & PAU, B. (1974) Propriètès d'une ATPase Ca^{2+} ou Mg^{2+} dépendate des membranes plasmiques de lymphocytes. Effect de la concanavaline A sur les ATPases membranaires. *Biochimie*, **56,** 1425.

DUPUS, G. & DOUCET, J.P. (1981) Electrophoretic studies of protein constituents of pig spleen lymphocyte plasma membrane and of a nonionic detergent extract of intact cells. *Biochim. Biophys. Acta*, **669,** 157.

ELLEGAARD, J. & DIMITROV, N.V. (1972) ATP-ase activity of lymphocytes from normal individuals and patients with cancer. *Cancer*, **30,** 881.

ERSOY, F. & BERKEL, A.I. (1974) Clinical and immunological studies in twenty families with ataxia–telangiectasia. *Turk. J. Pediatrics*, **16,** 145.

HO, N.N.Y. (1979) Direct autoradiography of cylindrical gels, introducing a simple longitudinal gel slicer. *Anal. Biochem.* **97,** 51.

KRAMERS, M.T.C., CATAVSKY, D. & FOA, R. (1978) Cell membrane enzymes: II. Alkaline phosphatase and alkaline phosphodiesterase I in normal and leukaemic lymphocytes. *Br. J. Haematol.* **40,** 111.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265.

MARCHALONIS, J.J., CONE, R.E. & SANTER, V. (1971) Enzymic iodination. A probe for accessible surface proteins of normal and neoplastic lymphocytes. *Biochem. J.* **124,** 921.

MICHELL, R.H. & HAWTHORNE, J.N. (1965) The site of disphosphoinositide synthesis in rat liver. *Biochem. Biophys. Res. Commun.* **21,** 333.

MURNANE, J.P. & PAINTER, R.B. (1983) Altered protein synthesis in ataxia–telangiectasia fibroblasts. *Biochemistry*, **22,** 1217.

NEUMANN, H., KLEIN, E., HAUCK-GRANOTH, R., YACHNIN, S. & BEN-BASSAT, H. (1976) Comparative study of alkaline phosphatase activity in lymphocytes, mitogen-induced blast, lymphoblastoid cell lines, acute myeloid leukemia and chronic lymphatic leukemia cells. *Proc. Natl. Acad. Sci. USA*, **73,** 1432.

PAINTER, R.B. & YOUNG, B.R. (1980) Radiosensitivity in ataxia–telangiectasia: a new explanation. *Proc. Natl. Acad. Sci. USA*, **77,** 7315.

PARKMAN, R., REMOLD-O'DONNEL, E., KENNEY, D.M., PERRINE, S. & ROSEN, F.S. (1981) Surface protein abnormalities in lymphocytes and platelets from patients with Wiskott–Aldrich syndrome. *Lancet*, **ii,** 1387.

PATERSON, C.M. & SMITH, P.J. (1979) Ataxia telangiectasia: an inherited human disorder involving hypersensitivity to ionizing radiation and related DNA-damaging chemicals. *Ann. Rev. Genet.* **13,** 291.

PAU, B., DORNAND, J. & MANI, J.C. (1976) Etude cinètique de l'activitè ATPases activèe par Ca^{2+} ou Mg^{2+} des membranes plasmiques lymphocytaires. *Biochimie*, **58,** 593.

PETTEGREW, J.W., NICHOLS, J.S. & STEWART, R.M. (1981) Membrane studies in Huntington's disease: steady state and time-dependent fluorescence spectroscopy of intact lymphocytes. *J. Neurochem.* **36,** 1966.

REMOLD-O'DONNEL, E., KENNEY, D.M., PARKMAN, R., CAIRNS, L., SAVAGE, B. & ROSEN, F.S. (1984) Characterization of a human lymphocyte surface sialoglycoprotein that is defective in Wiskott–Aldrich syndrome. *J. exp. Med.* **159,** 1705.

ROHLF, F.J. & SOKAL, R.R. (1969) In *Statistical Tables*, p. 240. W.H. Freeman and Co., San Francisco.

SETLOW, R.B. (1978) Repair deficient human disorders and cancer. *Nature*, **271,** 713.

SHERIDAN, R.B. & HUANG, P.C. (1979) Superoxide dismutase and catalase activities in Ataxia telangiectasia and normal fibroblast cell extracts. *Mutat. Res.* **61,** 381.

SMITH, W.I., LADOULIS, C.T., MISRA, D.N., GILL, T.J. & BAZIN, H. (1975) Lymphocyte plasma membranes. III. Composition of lymphocyte plasma membranes from normal and immunized rats. *Biochim. Biophys. Acta*, **382,** 506.

TAMURA, T., CHIBA, S., ABO, W., CHIBA, Y. & NAKAO, T. (1980) Cytomegalovirus-specific lymphocyte transformations in subjects of different ages with primary immunodeficiency. *Infect. Immun.* **28,** 49.

THOMPSON, R.A., CANDY, D.C.A. & MCNEISH, A.S. (1984) Familial defect of polymorph neutrophil phagocytosis associated with absence of surface glycoprotein antigen (OKMI). *Clin. Exp. Immunol.* **58,** 229.

WEBER, K., PRINGLE, J.R. & OSBORN, M. (1972) Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. In *Methods in Enzymology* (ed. by C.H.W. Hirs & S.N. Timashef) p. 3. Academic Press, New York.

WEISBART, R.H., KACENA, A., GOLDE, D.W. & COSTEA, N.V. (1980) Impaired cellular interactions involving lymphocytes from patients with chronic lymphocytic leukemia and ataxia–telangiectasia. *J. lab. clin. Med.* **96,** 114.

WILLIAMS, G.T., JOHNSTONE, A.P., BOURIOTIS, V. & DEAN, P.D.G. (1981) Affinity chromatography of membrane proteins on dihydroxyborylmatrix gel. *Biochem. Soc. Trans.* **9,** 137.