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Two Clonal Cell Populations (Mosaicism) in a 46,XY Male with Mucolipidosis II (I-Cell Disease)—An Autosomal Recessive Disorder

GEORGE H. THOMAS,[1,2] CAROL S. MILLER,[2] KATHLEEN E. TOOMEY,[3,4] LINDA W. REYNOLDS,[2] MARC L. REITMAN,[5] AJIT VARKI,[5] ANN VANNIER,[5] KENNETH N. ROSENBAUM,[3,4] WILMA B. BIAS,[6] AND BRIAN H. SCHOFIELD[7]

SUMMARY

Cultured fibroblasts from a 46,XY male with an atypical form of mucolipidosis II (I-cell disease) had two distinct phenotypes. One population of these fibroblasts had the morphological and biochemical features characteristic of I-cell disease, while the remaining cells were indistinguishable from normal fibroblasts. Direct evidence that the patient was a mosaic, having two cell populations, was provided by the establishment of pure, stable clones of both wild type and I-cell fibroblasts from each of two biopsies obtained several months apart. Additionally, it was shown that the I-cell fibroblasts lacked UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminylphosphotransferase while the morphologically normal cells contained levels of this enzyme just below or at the lower end of the normal range.

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[1] Departments of Pediatrics and Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

[2] The John F. Kennedy Institute, 707 N. Broadway, Baltimore, MD 21205.

[3] Children's Hospital National Medical Center, 111 Michigan Ave., N.W., Washington, DC 20010.

[4] Department of Child and Health and Development, George Washington University School of Medicine, Washington, D.C.

[5] Departments of Internal Medicine and Biological Chemistry, Washington University, School of Medicine, St. Louis, MO 63110.

[6] Department of Medicine, The Johns Hopkins University School of Medicine.

[7] Department of Orthopedic Surgery, The Johns Hopkins University School of Medicine.

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INTRODUCTION

Mucolipidosis II (I-cell disease) and mucolipidosis III (pseudo-Hurler polydystrophy) are autosomal recessive disorders. The former is characterized by coarseness of features, severe skeletal dysplasia, and marked psychomotor retardation [1], while the latter is a milder disorder having slight coarseness of features, mild mental retardation, and some skeletal abnormalities [2].

Cultured fibroblasts from patients with either of these disorders are characterized by the presence of dense, cytoplasmic inclusions, decreased lysosomal enzymes, and altered metabolism of mucopolysaccharides and sialic acid [3–6]. These alterations appear to be the consequence of the inability of these cells to generate the phosphomannosyl recognition signal believed to be required for receptor-mediated targeting of acid hydrolases [7]. Recent data indicate that the lack of the recognition marker is due to the deficiency of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminylphosphotransferase activity [8, 9].

We describe here a patient who has a clinically atypical form of one of these disorders and two distinct fibroblast types. We conclude that two lines of cells, one normal and one indistinguishable from I-cells, coexist in this patient and are the basis for his clinically atypical form of mucolipidosis. While a precise explanation for these findings is lacking, we suggest that cellular mosaicism may represent an unrecognized cause of genetic heterogeneity underlying some of the clinical variability found in autosomal recessive disorders.

CASE REPORT

The patient was the product of an uncomplicated, full-term pregnancy of a 20-year-old, healthy, gravida 2, para 1 female and her 23-year-old, nonconsanguineous mate. At birth he weighed 3.3 kg and was not obviously abnormal. At age two months, he underwent repair of bilateral inguinal hernias at which time thoraco-lumbar kyphosis was noted. The child was not seen again until age 10 months when he was also noted to have mild coarsening of facial features with significant gingival hyperplasia (fig. 1). Height and weight were at the 90th and 10th percentiles, respectively, with head circumference at the 5th percentile. Ophthal-

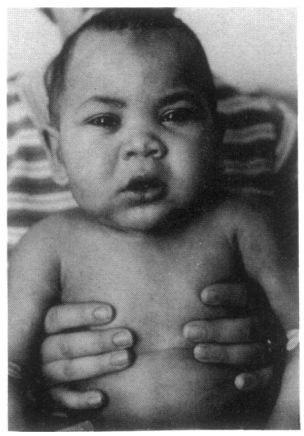

FIG. 1.—Patient at 10 months showing mild coarsening of facial features

mologic examination revealed normal fundi with no evidence of corneal clouding. There was no hepatosplenomegaly, and the genitalia were normal. The skin and subcutaneous tissues were thickened and smooth. Hair and nails were normal. A mild decrease in extension of both elbows and distal interphalangeal joints as well as an easily dislocatable left knee and hip were noted. Neurological exam was within normal limits.

A Bayley Infant Scale performed at age 12 months yielded a motor score of 76 and a mental score of 52, with most skills at 7 months scattering up to 10 months. Auditory and visual-evoked responses were normal except for a nonspecific, mild, left-right asymmetry in the long-latency components. Additional investigations including cranial CT scan, renal ultrasound, electrocardiogram, echocardiogram, CBC, and chromosomal analysis, and urine metabolic screening for abnormal components including mucopolysaccharides (glycosaminoglycans) were normal. Radiologic studies done at age 10 months revealed dysostosis multiplex with small, deformed, vertebral bodies of T12-L1, mild metaphyseal flaring, broad ribs, proximal pointing of the metacarpals and abnormal ilia with flattened, acetabular roofs, and subluxation of the hips. Repeat studies at age 14 months showed progression of these changes (fig. 2).

METHODS

Fibroblast cultures were established from skin biopsies obtained from the patient, his parents, and controls as described [3, 10]. All investigations on both the uncloned and cloned fibroblasts were performed on low-passage, confluent cultures, harvested 7–10 days after subculture. Clones were obtained from cultures of the patient according to the technique of Ham and Puck [11]. Cells of the uncloned cultures, after dissociation with trypsin, were plated (approximately 10 cells per 35-cm^2 culture dish) and cultured, and clones were plucked from well-isolated colonies after approximately 2 weeks. Following isolation, the clonal colonies were resuspended in individual 35-cm^2 tissue culture dishes and grown to confluence.

Phase and dark-field microscopy studies of the cloned and uncloned cells were performed as described by Taylor et al. [3]. Cell pellets for electron microscopy were fixed for 1.5 hrs at 4°C in 1.5% cacodylate-buffered glutaraldehyde containing 2.5 mM calcium chloride and 0.25% tannic acid. After washing overnight in .075 M cacodylate buffer containing 0.33 M

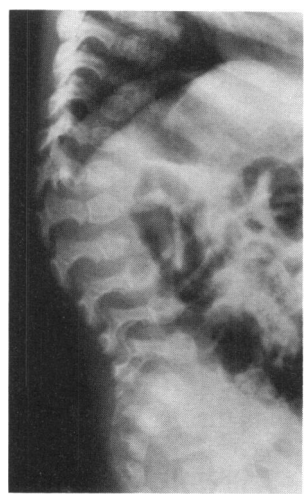

FIG. 2.—Features of dysostosis multiplex at 14 months including broad ribs and deformed vertebral bodies.

sucrose, the cells were post fixed in 1% osmium tetroxide, pH 7.2, for 20 min at 4°C, dehydrated in graded ethanols, and embedded in Epon 812. Thin sections were stained with lead citrate and examined by electron microscopy. Lysosomal enzyme activities were measured according to the methods of Thomas et al. [5, 12]. Neuraminidase (sialidase) was quantitated by the method of Myers et al. [13]. Total neuraminic acid levels were measured by a micro modification of the method of Warren [14]. $^{35}SO_4$ incorporation and chase studies were carried out according to a slight modification [12] of the method of Fratantoni et al. [15]. UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase activity was measured using both endogenous [9] and exogenous acceptors [16, 17]. Analysis of blood groups and serum types was carried out as described [18].

RESULTS

Initial microscopic examinations of fibroblasts cultured from the patient's forearm revealed two distinct cell phenotypes. When examined by phase or dark-field microscopy, 20%–25% of the cells had a very abnormal appearance (i.e., numerous coarse, granulated inclusions throughout the cell cytoplasm). The remaining cells (approximately 75%–80%) were indistinguishable from normal fibroblasts. A repeat biopsy taken 4 months later from a different site yielded identical results, except that the percentage of abnormal cells was higher (approximately 50%–60%).

Direct evidence for the presence of two populations of cells was obtained by cloning fibroblasts from both biopsies according to the method of Ham and Puck [11]. The cloning efficiency of cells obtained from each biopsy was approximately 30%. Of the isolated clones obtained from the 6th passage of biopsy 1 and the first passage of biopsy 2, 4% and 46%, respectively, were abnormal.

When the isolated clonal colonies were examined by phase and dark-field microscopy, two distinct cell types were found. All of the fibroblasts in the abnormal clones (no. = 10) were characterized by the presence of cytoplasmic inclusions of the type noted in 20%–60% of the cells in the original uncloned cultures (see fig. 3*a* and *c*). In the remaining clones (no. = 23), all the cells were phenotypically normal (fig. 3*b* and *d*). By electron microscopy, the cytoplasmic inclusions of the abnormal cells appeared to be membrane-limited structures (1–3 μm in size) containing pleomorphic membranes, as well as homogeneous, electron-dense material (fig. 4) of the type described in fibroblasts of I-cell patients [3]. Myelin figures were only infrequently observed. In addition, most of the abnormal cells contained an increased number of glycogen particles. Moreover, these particles were present in cytoplasm as well as in the membrane-limited structures (fig. 4).

Additional evidence that the cytoplasmic inclusions were associated with or resulted from lysosomal dysfunction of the type found in mucolipidoses II or III was obtained by examining the activities of a number of lysosomal enzymes. As shown in figure 5, cells of 10 abnormal clones had β-galactosidase, arylsulfatase A, and hexosaminidase levels that were, respectively, 2.9%, 6.8%, and 11.3% of the average value of 23 normal clones. Additionally, as shown in figure 6, these cells lacked sialidase (0.8% of the normal clones) and had a 3.2-fold increase in total sialic acid. The enzyme values of the phenotypically normal cells were indistinguishable form normal control fibroblasts (fig. 5). Finally, as shown in table 1, the measurement of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucos-

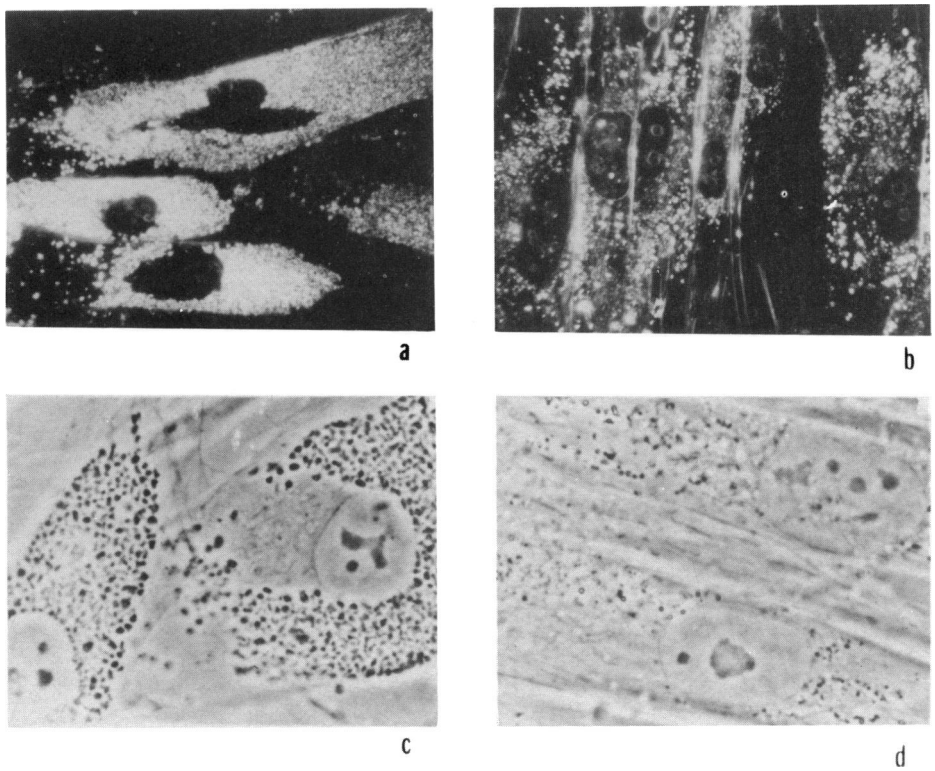

FIG. 3.—Appearances of living cultured fibroblasts of a normal and an abnormal clone. *a*, Abnormal clone by dark-field microscopy; *b*, normal clone by dark-field microscopy; *c*, abnormal clone by phase-contrast microscopy; and *d*, normal clone by phase-contrast microscopy.

amine-1-phosphotransferase using both exogenous and endogenous acceptors demonstrated that the abnormal clones lacked this activity while the normal clones had activities expected for the heterozygous state [19].

Additional evidence that the fibroblasts from the patient consisted of two clonal populations was provided by radioactive sulfate ($^{35}SO_4$) incorporation studies. The abnormal clonal colonies (no. = 10) accumulated an average of 3.1 times (range 2.3–4.2) as much $^{35}SO_4$ as did the normal clones. The latter were, in turn, indistinguishable from normal cells.

Fibroblasts cultured from the patient's parents lacked the structural or biochemical alterations found in their son's cells. There was no evidence of any clonal subtypes in these cultures. The UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase activity of the mother's fibroblast cells was, however, below the normal range with both endogenous and exogenous acceptors (table 1). In contrast, the father's values were close to the mean values of the normal control cells (table 1).

As is the case with mucolipidoses II and III, the patient's serum lysosomal enzymes were markedly elevated; for example: *N*-acetyl-β-glucosaminidase (17.5 $\times$

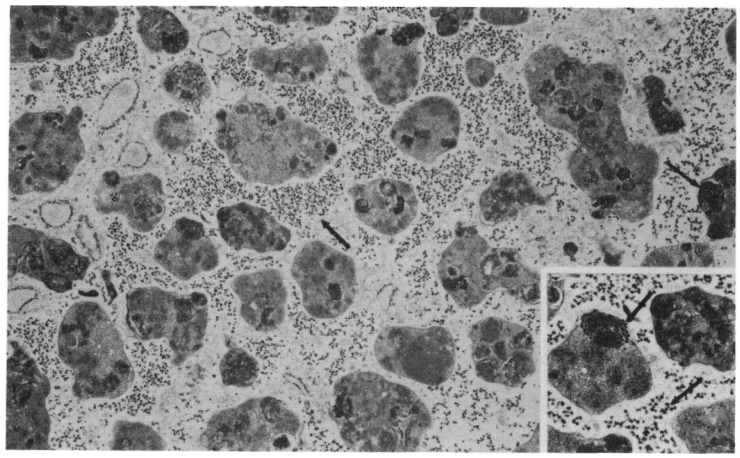

FIG. 4.—Appearance of cultured fibroblasts of an abnormal clone upon examination by electron microscopy. Note inclusions containing pleomorphic membranes and electron dense material as well as presence of glycogen-like storage material throughout cytoplasm (× 8,000). *Insert* shows vesicles and amorphous electron dense granule contents surrounded by glycogen particles. *Arrows* indicate glycogen.

normal), β-galactosidase (5.5 × normal), and arylsulfatase A (55.7 × normal). In the mother's and father's serum, the total hexosaminidase was, respectively, 2.1 and 1.3 × the normal mean. Moreover, the percentage of the heat-labile form (HEX A) of this enzyme was decreased (34% and 43% of the total activity vs. a normal average of 56% ± 6%). Finally, 51%, 49%, and 48% of the total hexosaminidase in the mother's, father's, and the patient's white blood cells was heat labile (normal value 63% ± 6%).

Typing of genetic markers on blood samples from the patient and his parents failed to demonstrate any evidence for unexpected or anomalous patterns of inheritance for HLA, a variety of red cell antigens, red cell enzymes, and serum proteins (table 2). In particular, the patient's samples were carefully scrutinized for evidence of two cell populations, and none was detected. Moreover, the electrophoretic patterns of esterase D and acid phosphatase of the normal and the abnormal clones were identical.

DISCUSSION

The clinical and laboratory findings described here are almost certainly the consequence of the coexistence, in the patient, of two distinct cell types. Moreover, the absence of cells having an intermediate phenotype and the presence of the two distinct cell types in close proximity, in cultures containing a mixed population of the two cell types, indicates that metabolic cross-correction does not occur. Evidence for clonal continuity (i.e., stable and pure phenotypes) is provided by the observation that the isolated clonal colonies could be subcultured for prolonged periods (at least 10–12 passages) without evidence of reversion to the alternate phenotype. Of the 10 abnormal clones, however, one (clone 69) was found to yield

intermediate results (table 1) soon after the cloning step. We conclude that this could have been the result of a failure to obtain a pure clone or, alternatively, the result of a new mutational event. Attempts to identify two cell populations or to further subclone this cell line, however, were unsuccessful. No other such occurrence was detected in any of the remaining 32 abnormal or normal clones over periods of 30–50 days in culture.

By both morphological and biochemical criteria, the abnormal cells are indistinguishable from cultured fibroblasts from mucolipidoses II or III patients. The absence of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminylphosphotransferase in these cells also directly supports this conclusion as this may be the primary enzyme defect in I-cell disease [8, 9]. Moreover, these laboratory findings are compatible with the clinical impression that the patient has an atypi-

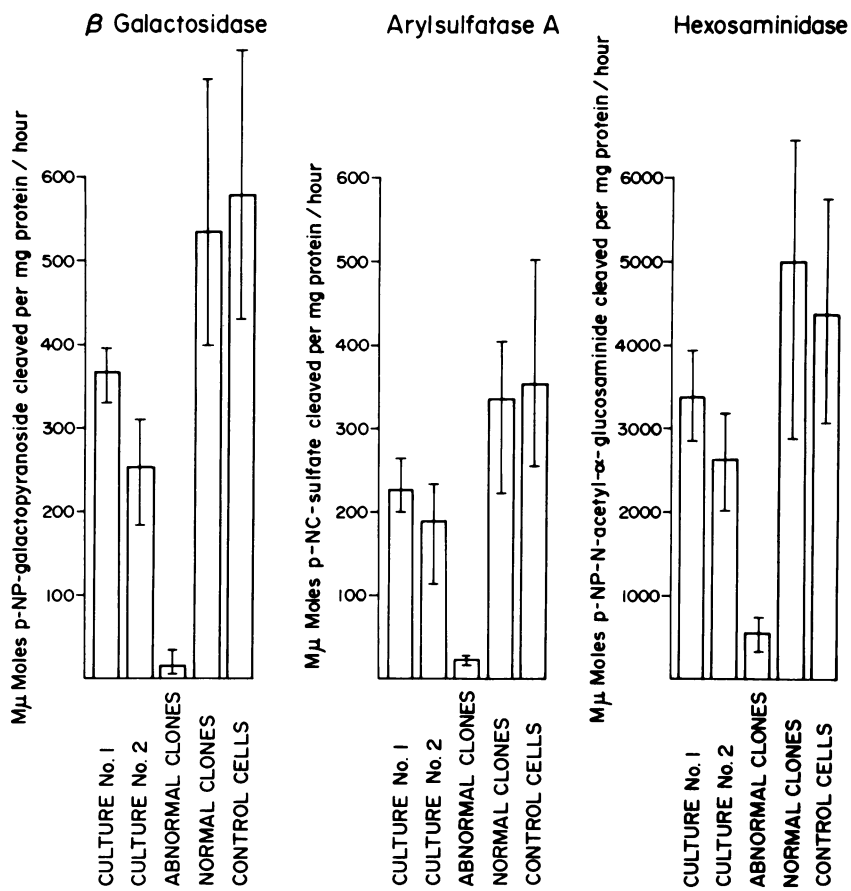

FIG. 5.—Average β-galactosidase, arylsulfatase A, and hexosaminidase levels of cultured fibroblast samples obtained from patient. *From left to right*, these are uncloned cultures from biopsy 1 (no. = 2), uncloned culture from biopsy 2 (no. = 3), abnormal clones from biopsies 1 and 2 (no. = 10), normal clones from biopsies 1 and 2 (no. = 23), and normal fibroblast controls (no. = 20).

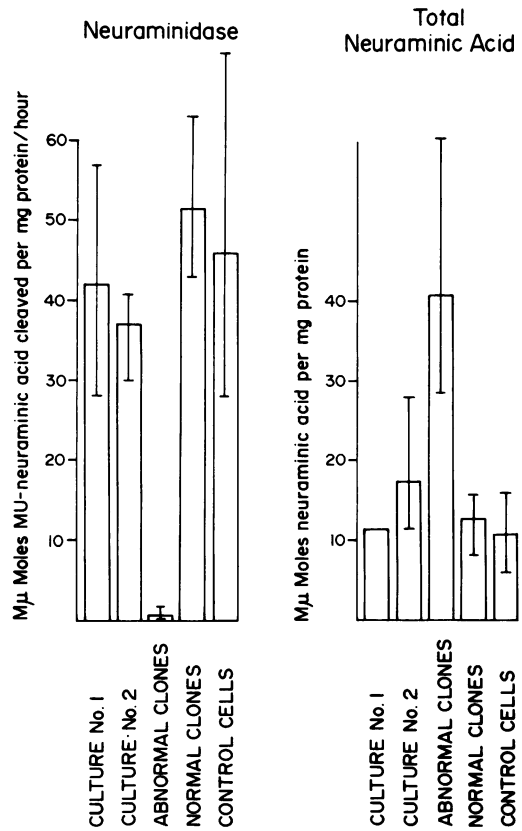

FIG. 6.—Average neuraminidase and total neuraminic acid of cultured fibroblast samples obtained from patients. See figure 5 for details of cell lines.

cal form of mucolipidosis that is too mild to be classified as mucolipidosis II and too severe to be considered mucolipidosis III. On the basis of these observations, we conclude that the patient is mosaic for mucolipidosis II and wild-type cells.

In view of the fact that mucolipidosis II is an autosomal recessive disorder and the patient's karyotype is 46,XY, the basis for the above findings is difficult to explain. A review of the medical literature reveals a number of reports concerning similar phenomena in other autosomal recessive disorders. German et al., for example, found both normal and abnormal blood lymphocytes to coexist in five of 21 patients with Bloom syndrome [20]. In these patients, the abnormal cells were characterized by an increased number of chromosomal gaps, breaks, and interchanges, while the remaining cells were indistinguishable from normal. Similarly, Auerbach et al. presented evidence for the presence of both normal and abnormal lymphocyte populations in a patient with Fanconi anemia [21]. Finally, several cases of apparent mosaicism affecting the ABO blood groups have been reported [22].

Possible explanations that have been suggested or cited to explain the above findings include twin chimerism, somatic mutation, somatic sister chromatid exchange, somatic chromosomal deletion or translocation, change in gene regulation, gametic half-chromatid mutation, and, in the case of nonhuman models, transposable elements.

Genetic marker studies of blood cells as well as a search for discordant chromosomal polymorphisms (by C-banding techniques) in the patient (blood and fibroblasts) and his parents failed to provide any evidence for twin chimerism in this patient. Chromosomal studies also failed to demonstrate any detectable chromosomal deletion or translocation in blood and/or tissue culture cells of the patient.

Obligate heterozygotes for mucolipidoses II and III have recently been found to have intermediate levels of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminylphosphotransferase [19]. The enzyme levels in the patient's normal clones and his mother's cells fall within this range (table 1). This suggests that one abnormal gene could have been contributed by the mother to both the normal and the abnormal clones. The enzyme levels in the father's cells were within the normal range; however, for reasons given elsewhere, we cannot conclusively rule out his being a carrier for the same defect [19]. Carriers for mucolipidoses II and III also have been found to have a nonspecific, but significant, increase in serum hexos-

TABLE 1

UDP-*N*-ACETYLGLUCOSAMINE:LYSOSOMAL ENZYME *N*-ACETYLGLUCOSAMINE-1-PHOSPHOTRANSFERASE ACTIVITY IN THE FIBROBLASTS OF THE PATIENT AND HIS PARENTS

Fibroblasts	Type	Exogenous acceptor	Endogenous acceptors
Patient:			
Culture #1	Uncloned	121	0.48
Culture #2	Uncloned	88	0.37
Clone #62	Abnormal	1.8	0.03
Clone #69	Abnormal	33.8	0.08
Clone #80	Abnormal	1.0	0.02
Clone #66	Normal	123	0.57
Clone #70	Normal	128	0.53
Clone #72	Normal	102	0.43
Father:			
Culture	Uncloned	185	0.77
Mother:			
Culture	Uncloned	111	0.40
Heterozygotes mucolipidosis II*:			
Mean		106	0.45
Range		53–140	0.26–0.87
Normal controls* (no. = 12):			
Mean		155	0.78
Range		130–186	0.45–1.03

NOTE: pmol *N*-acetylglucosamine-[^{32}P]phosphate transferred per mg protein/hr.
* Values taken from [19].

TABLE 2

LEUKOCYTE, RED CELL, AND SERUM MARKERS OF PATIENT AND HIS PARENTS

System	Father	Mother	Child
ABO	0	B	B
Rh	DcEe	DCe	DCcEe
MNSs	MsNs	MSNs	MSNs
Kell	neg	neg	neg
Penny	neg	neg	neg
Kidd	Jk (a+b−)	Jk (a+b+)	Jk (a+b+)
Duffy	Fy (a−b−)	Fy (a−b−)	Fy (a−b−)
P_1	P_1+	P_1+	P_1+
Xg^a	Xg (a−)	Xg (a+)	Xg (a+)
Sf^a	Sf (a−)	Sf (a+)	Sf (a+)
Lewis	Le (a−b+)	Le (a−b−)	Le (a−b+)
ADA	1-1	1-1	1-1
AK	1-1	1-1	1-1
AcP	AB	AB	AB
ESD	1-1	2-1	2-1
G6PD	B	BB	B
GPT	2-1	2-1	2-2
PGM (first locus)	1-1	1-1	1-1
PGM (second locus)	1-1	1-1	1-1
6PGD	AA	AA	AA
UMPK	1-1	1-1	1-1
Glyoxalase-1	2-1	2-1	2-1
Hp	2-1	2-1	2-1
Tf	cc	cc	cc
Gc	1-1	2-1	2-1
Gm*	1	1,3,5,13	1,3,5,13
HLA	a/b AW34,CW4,BW44,BW4 / AW32,CWX,BW53,BW4	c/d A3,CW3,BW62,BW6 / A28,CWY,BW49,BW4	a/c

* Gm types tested: Gm(1), Gm(2), Gm(3), Gm(5), Gm(6), Gm(13).

aminidase and an associated decrease in the percentage of the heat-labile form of this enzyme in both serum and white blood cells [2]. It is, thus, perhaps of significance that similar findings were obtained in the serum and white blood cells of each of the patient's parents.

At present, we are unable to identify the alteration that resulted in the coexistence of two distinct cell phenotypes in this patient. We do suspect, however, that, whatever the mechanism, it may be more common than heretofore believed. This suspicion is based, in part, on the fact that such mosaicism would be detected only in those situations in which the cellular pathology could be detected in individual cells (i.e., gross changes in cellular morphology, chromosomal structure, tissue antigens, etc.). As noted above, of the relatively small number of autosomal recessive disorders in which such changes could be detected, several examples of mosaicism have been detected. It is thus possible that a similar phenomenon may be responsible for some of the clinical variability encountered in this as well as other autosomal recessive disorders.

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