Table 1. Genotypic and phenotypic characteristics of patients A, B and C.

Pt.	Sex/ Age (y)	FVII:C	FVII:Ag	allele 1	FVIIR	allele 2	FVIIR	Polymorphic sites	Clinical features
A	F/62	<1%	64%	100Gln→Arg	+	331Gly→Ser	++	A1A1 - M1M1	asymptomatic
B	F/46	<1%	8%	100Gln→Arg	+	97Gly→Cys	+	A1A1 - M1M2	epistaxis, menorrhagia
C	F/52	<1%	7%	100Gln→Arg	+	49Gln→Stop	0	A1A1 - M1M1	recurrent hemarthrosis

The FVII coagulant activity (FVII:C) was assayed by a one-stage method based on the prothrombin time using a recombinant human tissue factor (Instrumentation Laboratory, Lexington, USA). The FVII antigen (FVII:Ag) was determined by an enzyme-linked immunoadsorbant assay using the Asserachrom FVII:Ag Kit (Diagnostica Stago, Asnière-sur-Seine, France). The FVII genotypes were characterized by direct sequencing as previously described.[5] A1 and A2 correspond to the presence or absence, respectively, of the 10 base-pair insertion at –323 in the promoter region of FVII gene . M1 and M2 correspond to the 323Arg and 353Gln alleles, respectively. FVIIR denotes the "presumed residual amount of FVII".

well as the previously described 331Gly→Asp mutation.[7]

In addition, as the FVII antigen level remains normal, the FVII 331Ser mutant protein seems to continue to be secreted. Together, these data are consistent with the hypothesis that there is a gradual decrease in the production of functional FVII protein from the 331Gly→Ser substitution, to the 97Gly→Cys mutation and to the 49Gln→Stop nonsense mutation. It is tempting to assume that this gradient of severity explains the different phenotypic expressions observed in the three probands.

Furthermore, the severity of bleeding could be related to the amount of FVII or activated FVII that is still produced. Thus, we suggest that a very small amount of FVII is sufficient to prevent the occurrence of a severe bleeding phenotype and that conventional FVII:C measurement fails to differentiate this gradual decrease in residual FVII activity.

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References

1. Mariani G, Lo Coco L, Bernardi F, Pinotti M. Molecular and clinical aspects of factor VII deficiency. Blood Coagul Fibrinolysis 1998; 9 Suppl 1:S83-8.
2. McVey JH, Boswell E, Mumford AD, Kemball-Cook G, Tuddenham EG. Factor VII deficiency and the FVII mutation database. Hum Mutat 2001; 17:3-17.
3. Kemball-Cook G, Jonhson DJ, Takamiya O, Banner DW, McVey JH, Tuddenham EG. Coagulation factor VII Gln100→Arg. Amino acid substitution at the epidermal growth factor 2-protease domain interface results in severely reduced tissue factor binding and procoagulant function. J Biol Chem 1998; 273:8516-21.
4. Hunault M, Arbini AA, Carew JA, Peyvandi F, Bauer KA. Characterization of two naturally occuring mutations in the second epidermal growth factor-like domain of factor VII. Blood 1999; 93:1237-44.
5. Giansily-Blaizot M, Aguilar-Martinez P, Biron-Andreani C, Jeanjean P, Igual H, Schved JF, et al. Analysis of the genotypes and phenotypes of 37 unrelated patients with inherited factor VII deficiency. The Study Group of Factor Seven Deficiency. Eur J Hum Genet 2001; 9:105-12.
6. Kumar A, Blumenthal DK, Fair DS. Identification of molecular sites on factor VII which mediate its assembly and function in the extrinsic pathway activation complex. J Biol Chem 1991; 266:915-21.
7. Zheng DQ, Shurafa M, James HL. Factor VII G331D: a variant molecule involving replacement of a residue in the substrate-binding region of the catalytic domain. Blood Coagul Fibrinolysis 1996; 7:93-6.

A novel case of immunodeficiency, centromeric instability, and facial anomalies (the ICF syndrome): immunologic and cytogenetic studies

The immunodeficiency, centromeric instability, and facial anomalies (ICF) syndrome is characterized by hypogammaglobulinemia and recurrent bacterial infections. Here we report a novel case of ICF syndrome with hypogammaglobulinemia and an inverted CD4/CD8 ratio. Cytogenetically abnormal cells, that were identified in both $CD4^+$ and $CD4^-$ peripheral blood lymphocytes, retained their ability to proliferate *in vitro* following polyclonal stimulation. A primitive defect of B-cell differentiation was detected.

The ICF syndrome is a rare autosomal recessive disorder;[1-3] to date, approximately 20 cases have been reported. Patients with ICF syndrome suffer from recurrent respiratory and/or gastrointestinal infections, and have facial anomalies, as well as mental retardation of variable degree.[1-3] They display hypogammaglobulinemia involving two or more isotypes[1-5] and, rarely, lymphopenia or an inverted CD4/CD8 ratio.[4,5] Juxtacentromeric abnormalities involving chromosomes 1 and 16 and, to a lesser extent, chromosome 9 are the diagnostic hallmarks of the disease.[1-3] The majority of ICF cases display mutations in the DNA methyltransferase 3B (DNMT3B) gene.[6-8]

Here we report a new case of ICF syndrome with hypogammaglobulinemia and an inverted CD4/CD8 cell ratio.

The propositus, a male, was born in 1981 in a small village in the south of Italy. He is the second child of healthy non-consanguineous parents. The pregnancy and delivery at full term were uneventful, and the child's weight at birth was 2950 g. At the age of 3 months, the propositus manifested bronchiolitis and recurrent respiratory infections. He was hospitalized at the age of 14

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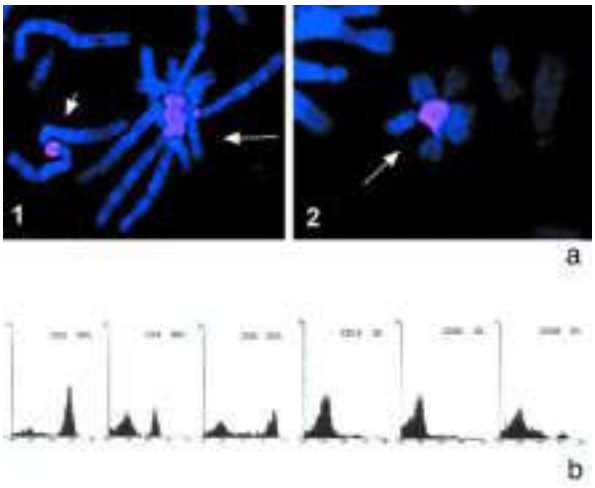

Figure 1a. FISH analysis of the patient's peripheral blood lymphocytes with the probes for satellite II of chromosome 1 or 16. Partial metaphases from phytohemagglutin-stimulated lymphocytes. The probes give rise to a red signal; chromosomes are counterstained with DAPI (blue). a1) A multibranched chromosome 1 fused with 16qh (as detected by chromosome banding) is shown together with a single fused block of heterochromatin labeled in red with the pMG1 probe specific for 1qh (arrow). The arrowhead indicates a normal chromosome 1. a2) Multibranched chromosome 16 labeled with the chromosome 16 specific heterochromatic probe pHUR195. Figure 1b. Flow cytometric analysis with monoclonal antibodies to the patient's peripheral blood lymphocytes.

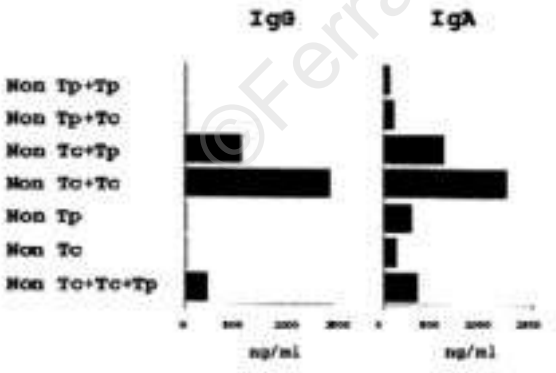

Figure 2. Pokeweed mitogen (PWM)-induced *in vitro* production of IgG and IgA. Peripheral blood MNC from the patient with ICF syndrome and an age-matched normal control were separated into T- and non-T-cells by E-rosetting and cultured in different combinations at a T-/non-T-cell ratio of 0.5:1 in the presence of PWM. Cultures were harvested after 7 days and supernatants were tested for the presence of IgG and IgA by ELISA. Results are expressed as ng/mL. p indicates the patient, c indicates the control subject.

months because of bronchopneumonia and measles. Hypogammaglobulinemia, hepatosplenomegaly and failure to thrive were found. A liver biopsy did not provide any diagnostic information and hepatomegaly was no longer detected in the subsequent clinical course of the patient.

The patient was first referred to our hospital at the age of 13 years because of chronic bronchopneumonia and bronchiectasis in the absence of hematologic abnormalities. Immunoglobulin serum levels were the following: IgM = 124 mg/dL (normal range for the age = 40-230 mg/dL), IgG = 380 mg/dL (normal range for the age = 550-1600 mg/dL), IgA = 20 mg/dL (normal range for the age = 40-230 mg/dL). His weight and height were 32 kg and 146 cm (10th centile), respectively; head circumference was 54 cm. He showed epicanthus, hypertelorism, a flat nasal bridge, low set ears, an asymmetrical thorax and slight splenomegaly (2 cm below the left costal margin). Mental retardation (IQ= 61) was also documented; at that time, the patient was attending the first class of secondary school. Cytogenetic studies supported the diagnosis of ICF syndrome. Associations and interchanges in the juxtacentromeric region among homologous or non-homologous chromosomes, deletions of whole arms, chromatids and isochromatids, breaks in the juxtacentromeric region and multibranched configurations formed by a variable number of arms of the same or different chromosomes were detected in peripheral blood mononuclear cells (MNC) stimulated with phytohemagglutin (PHA) for 72 h (Figure 1A).[1-3] In these experiments, 69/212 T-cell blasts displayed chromosomal abnormalities.

Interphase FISH analysis demonstrated that both CD4$^+$ and CD4$^-$ lymphocytes, freshly isolated by immunomagnetic bead manipulation, contained 92/400 and 25/200 cytogenetically abnormal cells, respectively.

Immunophenotypic analyses performed at different times, in the absence of active infectious or inflammatory disorders at least 2 weeks before and after testing, showed a stable inversion of the CD4/CD8 ratio (0.65 on average). Normal proportions of CD20$^+$ B-cells and of CD16$^+$, CD56$^+$ NK cells were found (Figure 1B).

In subsequent experiments, peripheral blood MNC were cultured in the presence of PHA and recombinant interleukin-2 (50 IU/mL) for 14 days and tested for cytogenetic abnormalities after separation into CD4$^+$ and CD4$^-$ cells.

It was found that 97/360 CD4$^+$ T-cell blasts were cytogenetically abnormal; of these, 71/97 showed deletions, duplications or chromosomal associations and 26/97 displayed multibranched configurations. Furthermore, 60/408 CD4$^-$ cell blasts were also cytogenetically abnormal, with 51/60 of them showing deletions, duplications or chromosomal associations and 9/60 displaying multibranched configurations.

Next, T and non-T (B-cell-enriched) cells were isolated from the peripheral blood of the patient and an age-matched control and co-cultured with pokeweed mitogen in different combinations.[9] Lower amounts of IgG and IgA were detected in supernatants from control B-cells co-cultured with the patient's T-cells than in those from control B-cells incubated with control T-cells (Figure 2). This finding may be related to the lower number of CD4$^+$ cells in the patient's T-lymphocytes, but defective B-cell help by such cells cannot be ruled out.

The patient's B-cells produced little IgG or IgA when cultured with either autologous or control T-cells. Finally, the patient's T-cells suppressed pokeweed mitogen-driven terminal differentiation of normal B-cells co-cultured with normal T-cells (Figure 2).

In conclusion, we have shown that, in this new case of ICF with hypogammaglobulinemia and a stably inverted CD4/CD8 cell ratio, cytogenetically abnormal T-cells were able to divide and clonally expand following *in vitro* stimulation, suggesting that such cells are functional *in vivo*. This finding fits with the unusual manifestations of defective cell-mediated immunity in patients

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with ICF syndrome. Finally, hypogammaglobulinemia was found to depend in part on a primary B-cell defect.

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References

1. Brown DC, Grace E, Sumner AT, Edmunds AT, Ellis PM. ICF syndrome (immunodeficiency, centromeric instability and facial anomalies): investigation of heterochromatin abnormalities and review of clinical outcome. Hum Genet 1995; 96:411-6.
2. Tiepolo L, Maraschio P, Gimelli G, Cuoco C, Gargani GF, Romano C. Multibranched chromosomes 1, 9, and 16 in a patient with combined IgA and IgE deficiency. Hum Genet 1979; 51:127-37.
3. Turleau C, Cabanis MO, Girault D, Ledeist F, Mettey R, Puissant H, et al. Multibranched chromosomes in ICF syndrome: immunodeficiency, centromeric instability, and facial anomalies. Am J Med Genet 1989; 32:420-4.
4. Franceschini P, Martino S, Ciocchini M, Ciuti E, Vardeu MP, Guala A, et al. Variability of clinical and immunological phenotype in immunodeficiency-centromeric instability-facial anomalies syndrome. Report of two new patients and review of the literature. Eur J Pediatr 1995; 154:840-6.
5. Gimelli G, Varone P, Pezzolo A, Lerone M, Pistoia V. ICF syndrome with variable expression in sibs. J Med Genet 1993; 429-32.
6. Hansen RS, Wijmenga C, Luo P, Stanek AM, Canfield TK, Weemaes CM, et al. The DNMT3B DNA methyltransferase gene is mutated in the ICF immunodeficiency syndrome. Proc Natl Acad Sci USA 1999; 96:14412-7.
7. Hansen RS, Stoger R, Wijmenga C, Stanek AM, Canfield TK, Luo P, et al. Escape from gene silencing in ICF syndrome: evidence for advanced replication time as a major determinant. Hum Mol Genet 2000; 9:2575-87.
8. Wijmenga C, Hansen RS, Gimelli G, Bjorck EJ, Davies EG, Valentine D, et al. Genetic variation in ICF syndrome: evidence for genetic heterogeneity. Hum Mutat 2000; 16:509-17.
9. Kuritani T, Cooper MD. Human B-cell differentiation. I. Analysis of immunoglobulin heavy chain switching using monoclonal anti-immunoglobulin M, G, and A antibodies and pokeweed mitogen-induced plasma cell differentiation. J Exp Med 1982; 155:839-51.

Second allogeneic hematopoietic stem cell transplantation in hematologic malignancies in children: long-term results of a multicenter study of the Spanish Working Party for Bone Marrow Transplantation in Children (GETMON)

Twenty-four children with acute leukemia (21) or chronic myeloid leukemia (3) who relapsed after a first hematopoietic stem cell transplantation (HSCT) underwent a second allogeneic HSCT. Sixteen patients died from relapse or transplant-related causes and 8 are alive and disease-free with a probability of event-free survival at 5 years of 32%. These results show that this procedure offers a chance to a subset of these patients.

Relapse is the most frequent cause of therapeutic failure in patients with hematologic malignancies undergoing allogeneic hematopoietic stem cell transplantation (HSCT).[1] The optimal treatment strategy for these patients remains an open question. Conventional chemotherapy (CT) can achieve complete but generally brief remission. Other treatment options are donor leukocyte infusions (DLI) or a second HSCT.[2] The results of GETMON in children with hematologic malignancies undergoing second allogeneic HSCT are reviewed.

From May 1985 to April 1998, 24 children (13 males, 11 females) with hematologic malignancies received a second HSCT. All patients were in complete clinical and hematologic remission (CR) achieved by conventional CT (acute leukemias) or in second chronic phase (chronic myeloid leukemia). The same HLA identical sibling donor was used for both transplants, except in two cases in which another HLA identical sibling was used. From 1985 to 1996, HLA typing of donors and transplant recipients was performed by serology and by DNA techniques thereafter.

There were 11 cases of acute lymphocytic leukemia (ALL), 10 of acute myeloid leukemia (AML), and 3 of chronic myeloid leukemia (CML). The median age at second transplantation was 9 years (range 2-16) (Table 1). Conditioning for the first HSCT consisted of fractionated total body irradiation (TBI) plus CT (18 cases) or CT alone (6 cases). At second transplant, 19 patients received CT alone and 5 received fractionated TBI plus CT. Four patients received fractionated TBI in both conditionings. Graft-versus-host disease (GvHD) prophylaxis at first HSCT was performed with cyclosporin A (CsA) at an initial dose of 5 mg/kg/day intravenously in two cases and CsA plus methotrexate (MTX) at a dose of 10 mg/kg on alternate days for a total of 4 days in the remaining cases. At second HSCT 19 patients received CsA at an intitial dose of 3 mg/kg/day intravenously and 5 received CsA plus MTX at the same standard dose as in the first HSCT (Table 2). Twenty-two patients achieved a stable graft at second transplantation. Eight patients presented acute GvHD grade 1-2 at first transplant and 7 developed acute GvHD > grade 1 (grade 3-4 in three patients) at second HSCT.

Eight of the 24 patients are alive and event-free at a median follow-up of 82 months (range 38-142). The probability of event-free survival (EFS) at 5 years was 32%. Eight of thirteen patients who relapsed more than 12 months post-HSCT are alive and event-free. All 11 patients who relapsed < 12 months post-HSCT have died ($p = 0.001$).

Sixteen patients died after their second HSCT: 8 from relapse, 8 from transplant-related causes [graft failure (2), acute GvHD (2), veno-occlusive disease (1) and interstitial pneumonia (3)]. The interval between transplants was less than 12 months in 7 of the 8 patients who died from toxicity. The three patients who died from interstitial pneumonia received fractionated TBI in both transplants. The two cases of graft failure had ALL and received an infusion of 2.5×10^6/kg and 5.0×10^6/kg $CD34^+$ cells,