Available online at www.sciencedirect.com
SCIENCE DIRECT®
Clinical Immunology 110 (2004) 22–29
CLINICAL IMMUNOLOGY
www.elsevier.com/locate/yclim

Clinical, immunologic and genetic analysis of 29 patients with autosomal recessive hyper-IgM syndrome due to Activation-Induced Cytidine Deaminase deficiency

Pierre Quartier,[a,1] Jacinta Bustamante,[a,1] Ozden Sanal,[b] Alessandro Plebani,[c] Marianne Debré,[a] Anne Deville,[d] Jiri Litzman,[e] Jacov Levy,[f] Jean-Paul Fermand,[g] Peter Lane,[h] Gerd Horneff,[i] Guzide Aksu,[j] Isik Yalçin,[k] Graham Davies,[l] Ilhan Tezcan,[b] Furgen Ersoy,[b] Nadia Catalan,[m] Kohsuhe Imai,[m] Alain Fischer,[a,m] and Anne Durandy[m,*]

[a] *Hôpital Necker-Enfants Malades, Unité d'Immunologie-Hématologie et Rhumatologie Pédiatrique, Paris, France*
[b] *Ihsan Dogramaci Children's Hospital, Division Hacettepe University, Ankara, Turkey*
[c] *Clinica Pediatrica and Instituto di Medicina Molecolare Angelo Nocivelli, Università di Brescia, Italy*
[d] *Fondation Lenval, Nice-France, France*
[e] *Department of Clinical Immunology and Allergology, Masaryk University, Brno, Czech Republic*
[f] *Department of Pediatrics, Soroka Medical Center, Ben Gurion University, Beer Sheva, Israel*
[g] *Departement d'Immuno-Hématologie, Hôpital Saint-Louis, Paris, France*
[h] *Centre for Immune Regulation, Medical School University, Birmingham, United Kingdom*
[i] *Universitätsklinik und Poliklinik für Kinder und Jugendmedizin, Martin-Luther Universität, Halle, Wittenberg, Germany*
[j] *Department of Pediatric Immunology, Ege University Hospital, Bornova, Izmir, Turkey*
[k] *Subdivision of Clinical Immunology, Department of Pediatrics, Istanbul University, Istanbul, Turkey*
[l] *Great Ormond Street Hospital for Sick Children, London, United Kingdom*
[m] *Hôpital Necker-Enfants Malades, INSERM U429, Paris, France*

Received 8 September 2003; accepted with revision 17 October 2003

Abstract

Mutations of the *Activation-Induced Cytidine Deaminase* (*AID*) gene have been found in patients with autosomal recessive hyper-IgM (HIGM) syndrome type 2. We retrospectively analyzed clinical, immunologic and genetic characteristics of 29 patients from 22 families with AID deficiency. Patients' median age at diagnosis and at last evaluation was 4.9 years (range: 0 to 53) and 14.2 years (range: 2.7 to 63), respectively. Most patients had suffered from recurrent and severe infections, however, intravenous immunoglobulin (IVIG) replacement therapy resulted in a dramatic decrease in the number of infections. Lymphoid hyperplasia developed in 22 patients and persisted in 7 at last follow-up. It is striking to note that six patients developed autoimmune or inflammatory disorders including diabetes mellitus, polyarthritis, autoimmune hepatitis, hemolytic anemia, immune thrombocytopenia, Crohn's disease and chronic uveitis. Fifteen distinct *AID* mutations were found but there was no significant genotype–phenotype correlation. In conclusion, AID-deficient patients are prone to infections and lymphoid hyperplasia, which may be prevented by early-onset IVIG replacement, but also to autoimmune and inflammatory disorders.

Keywords: AID; Immune deficiency; Child; Autoimmunity; Intravenous immunoglobulin

Introduction

Hyper-IgM (HIGM) syndrome is a primary immunodeficiency characterized by normal or elevated serum IgM levels together with an absence of IgG, IgA and IgE indicating a defective class switch recombination (CSR) process [1]. The X-linked form of the disease called HIGM1 is caused by mutations in the gene encoding CD40-Ligand (CD40-L,

* Corresponding author. Hôpital Necker-Enfants Malades, INSERM U429, 149, rue de Sèvres, 75 743 Paris CEDEX 15, France. Fax: +33-1-44-49-50-70.
E-mail address: durandy@necker.fr (A. Durandy).
[1] These authors equally contributed to this work.

1521-6616/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.clim.2003.10.007

CD154) [2–5]. CD40-L is expressed on activated helper T cells and its interaction with CD40, which is constitutively expressed on B cells, is required for full B-cell terminal differentiation in germinal centers of secondary lymphoid organs [6,7]. CD40 is also expressed on monocytes, dendritic cells and myeloid progenitor cells [8,9]. HIGM1 patients are thus not only prone to bacterial and enteroviral infections, in a similar way to other patients with severe B-cell deficiencies, but also to opportunistic infections mainly due to *Pneumocystis carinii* and *Cryptosporidium* as observed in patients with T-cell defects [10–13] and to neutropenic complications. Another form of X-linked HIGM syndrome associated with anhydrotic ectodermal dysplasia has been described secondary to missense mutations in the gene encoding nuclear factor kappa B (NF-κB) essential modulator (NEMO or IKKγ) which is required for CD40-induced activation of the transcription factor NF-κB [14–16].

Besides the defined HIGM syndromes with X-linked inheritance, other causes of HIGM have been described [17–20]. In most of the patients, the HIGM syndrome is clearly transmitted with an autosomal recessive inheritance pattern. Four patients in three consanguineous families have been reported as suffering from defective expression of CD40 molecules due to homozygous mutations in the *CD40* gene, resulting in an immunodeficiency comparable to that observed in HIGM1 patients [21,22]. Recently, a more frequent entity characterized by a selective CSR deficiency has been described as HIGM4 syndrome [23] and mutations of the uracyl-DNA-glycosylase UNG gene have been found in humans with autosomal recessive HIGM syndrome [24]. A more frequent form of autosomal recessive HIGM syndrome (HIGM2) has been related to mutations in the gene coding for the Activation-Induced Cytidine Deaminase (AID), a recently described molecule selectively expressed in vivo and in vitro CSR-induced B cells [25]. These patients present not only a CSR defect characterized by a lack of IgG, IgA and IgE production but also defective generation of somatic hypermutations (SHM) in the immunoglobulin variable region genes resulting in impaired antibody affinity maturation [26]. This observation, in keeping with the identical phenotype of AID-deficient mice [27], provides strong evidence for the essential role of AID in the major events of B-cell terminal differentiation occurring in germinal centers, that is, CSR and SHM generation. In an effort to better characterize the clinical and immunologic phenotype of HIGM2 patients and to analyze whether genotype–phenotype correlations could be found, we retrospectively analyzed a cohort of 29 patients with a diagnosis of AID deficiency.

Patients and methods

Patient selection and data collection

Between December 1999 and June 2002, 56 patients with HIGM syndrome, as defined by markedly diminished serum levels of IgG and IgA with normal or increased serum levels of IgM, normal CD40L cell surface expression and no CD40L gene mutation were tested for *AID* gene mutations. Thirty-one patients from twenty-three distinct families were diagnosed as carrying *AID* gene mutations. Detailed clinical data of 29 patients from 22 distinct families were available. Clinical and immunologic data had been reported 7 years ago before molecular diagnosis for one of these patient (Patient 2 in Table 1) [28]. In 18 other patients (see legend of Table 1), AID mutations have been more recently reported, however, no detailed clinical data were available [26]. For the present study, patients notes were retrospectively reviewed and information was collected using a questionnaire sent to the patients physicians. Information was collected on family history, age at disease onset and at HIGM syndrome diagnosis, clinical features before and after diagnosis, serum Ig levels at diagnosis, therapeutic features and clinical status at last follow-up. Informed consent was obtained from all patients or their parents.

Immunologic and genetic analyses

T (CD3+, CD4+, CD8+) and B (CD19+, CD40+) cell counts were measured in all patients by FACS analysis using specific monoclonal antibodies as previously reported [26]. Expression of CD27 molecules, a marker designated as specific for "memory" B cells [29], was also assessed on CD19+ B cells. CSR to IgE was tested by ELISA after a 12-day activation of peripheral blood lymphocytes by soluble CD40-L and recombinant IL4 [20].

The five exons of the *AID* gene and their flanking sequences were sequenced on genomic DNA using the previously described primers and PCR amplification conditions [26]. Southern blotting was also performed on genomic DNA in patients in whom the *AID* gene could not be amplified.

Results

Patient baseline characteristics

Patients were either of Caucasian (n = 28) or Asiatic (n = 1) ethnic origin. Out of 29 patients, 18 were from consanguineous families. In the other patients, the pedigrees were consistent with an autosomal recessive pattern of inheritance. In two cases, older brothers died from infections, including bacterial meningitis in the elder brother of Patient 14. Sex ratio was 22M/7F, likely due to a bias in patients' recruitment.

The median age at first clinical manifestation was 2.0 years (range: 0.3 to 12.9). The median age at diagnosis of immunodeficiency and at diagnosis of HIGM syndrome was 3.8 years range: 0 to 44.3) and 4.9 years (0 to 52.7), respectively.

Table 1
Patients characteristics before initiation of IVIG substitution

Pt no.	Sex	Origin	Age (years) at		Infections			Lymph hyperplasia	Other clinical features	Serum Ig levels (g/l)			*AID* mutations	AID Protein
			First sympt	IVIG onset	Recurrent URT	Recurrent LRT	Severe infections			IgG	IgA	IgM		
1	M	Italy	5	21	x	x	hepatitis B	x	Thrombocytopenia	NA	NA	NA	[ex1-ex5del] + [ex1-ex5del]	Deletion
2	M	France	<12	42[a]	x	x	pneum		polyarthritis[b]	1.1	0.1	11.1	[ex1-ex5del] + [ex1-ex5del]	Deletion
3	M	Turkey	2	12	x	x	pneum, *Giardia*	x		<0.02	<0.02	37	[21-39 del] + [21-39 del]	M7 fs X 10
4	M	Turkey	8	14.1	x	x	adenitis, pneum	x		<0.06	<0.02	7	[70C>T] + [70C>T]	R24W
5	M	Turkey	1	13	x	x	pneum, HSV encephalitis	x		<0.05	<0.02	17	[70C>T] + [70C>T]	R24W
6	F	Turkey	2.1	4.2	x		pneum		chronic diarrhea,	0.5	<0.07	1.6	[70C>T] + [70C>T]	R24W
7	F	Turkey	1.8	2	x		pneum		chronic diarrhea,	<0.02	<0.02	5.6	[70C>T] + [70C>T]	R24W
8	F	Germany	1.2	1.7	x			x	AIHA, ITP, AI hepatitis[c]	<0.4	<0.1	30	[166C>T] + [166C>T]	H56X
9	M	Morocco	2	2.8	x		pneum, mastoiditis	x	chronic diarrhea	<0.06	<0.07	4.5	[203G>A] + [ex1-5del]	W68X + deletion
10	M	Morocco	2	5	x		pneum, impetigo	x		<0.06	<0.07	1	[203G>A] + [ex1-5del]	W68X + deletion
11	M	Morocco	1	2.3	x	x		x		<0.06	<0.07	1	[203G>A] + [ex1-5del]	W68X + deletion
12	M	Morocco	<1	1	x	x			fever, diarrhea	0.4	<0.07	2.4	[203G>A] + [175-183del]	W68X + L59-F61 del
13	M	Morocco	0.9	0.9						<0.06	<0.07	1.5	[203G>A] + [175-183del]	W68X + L59-F61 del
14	M	Israel	<7	14.3[a]	x	x	pleuropneum skin abscess		sinus	0.1	0.1	14.4	[203G>A] + [203G>A]	W68X
15	M	Israel	2	9.8[a]	x	x	pneum, cellulitis		sinus	1.5	<0.06	3.2	[203G>A] + [203G>A]	W68X
16	M	Turkey	0.3	1.1	x	x	men			1.3	0.2	10	[238T>C] + [238T>C]	W80R
17	F	Czechia	0.5	8.7	x			x		<0.4	<0.05	9.35	[259T>C] + [259T>C]	C87R
18	F	Turkey	0.4	4		x	men, pneum, osteomyelitis	x	congenital hypothyroidism	0.1	<0.02	11	[317T>C] + [317T>C]	C106P
19	M	Turkey	9	12	x	x	men, pneum	x	aseptic arthritis	0.5	<0.02	10	[317T>C] + [317T>C]	C106P
20	M	Turkey	7	7.2	x	x	pneum, *Giardia*	x	chronic hepatitis, aseptic arthritis, sinus	0.05	<0.02	34	[415A>G] + [415A>G]	M139V
21	M	Turkey	0.7	1.7	x			x	sinus	NA	<0.23	8.47	[415A>G] + [415A>G]	M139V
22	M	Italy	0.3	0.7	x	x		x		0.21	0.06	0.87	[441C>A] + [441C>A]	C147X
23	F	Italy	0.8	5[a]	x	x	pneum		chronic diarrhea	0	0	8	[441C>A] + [441C>A]	C147X
24	M	Turkey	3	3.1	x	x	pneum	x	sinus	0.7	0.1	9	[452T>C] + [452T>C]	F151S
25	F	Turkey	4	7.7	x	x	men, pneum	x		<0.02	<0.02	11	[452T>C] + [452T>C]	F151S
26	M	Turkey	0.3	2.3	x	x	Pneum	x	bronchiectasis	0.05	0.07	0.71	[522A>C] + [522A>C]	R174S
27	M	Turkey	3	16.2[a]	x	x	pneum	x	Sinus, bronchiectasis	0.3	0	3.45	[522A>C] + [522A>C]	R174S
28	M	Turkey	<13	18	x	x	pneum	x		0	0	4.83	[522A>C] + [522A>C]	R174S
29	M	Pakistan	<3	3	x	x		x		0.1	0	5.5	[V5-2A>G] + [V5-2A>G]	L181L-31 ins-P182

Table 2
Severe or chronic infections before and after the initiation of IVIG therapy

Infections (description)	Before IVIG therapy		After initiation of IVIG	
	No. of patients	Median age [range] at IVIG initiation (years)	No. of patients	Mean time [range] on IVIG (years)
Severe infections				
Pneumonia	17	7.7 [2.3 – 42]	3	7.6 [5.6 – 10.4]
Meningitis	4[a]	5.9 [1.1 – 12]	0	
HSV encephalitis	1	13	0	
Other infections	6[b]	6.9 [2.8 – 14.3]	2[c]	14.9 [8.8 – 21]
Recurrent/chronic infections				
URT infections	27	6.2 [0.7 – 42]	11	7.6 [2.1 – 19]
Bronchitis	21	5.0 [0.7 – 42]	5	8.2 [3 – 19]
Aseptic arthritis	2	10.8 [9.6 – 12]	0	
Gastroenteritis	5[d]	7.3 [1.8 – 12]	3[d]	9.0 [4.8 – 12.7]
Chronic hepatitis B	1	24	0	

[a] *H. influenzae* in one case, no microorganism isolated in the three other patients.
[b] Severe skin/soft tissues infections, osteomyelitis, mastoiditis, bacterial adenitis.
[c] Arthritis in one case, sepsis resulting to death in the other, no microorganism identification.
[d] *G. lamblia* was found in two cases before IVIG therapy and in two other patients on IVIG.

Infections

Severe or recurrent infections occurred in all patients before intravenous immunoglobulin (IVIG) replacement was initiated (Tables 1 and 2). Upper respiratory tract (URT) infections and bronchitis were the most common features, followed by gastrointestinal tract infections. Central nervous system infections were diagnosed in five patients. One patient had *Haemophilus influenzae* meningitis, another patient Herpes simplex virus (HSV) encephalitis and three patients meningitis with no identified microorganism. Recurrent aseptic arthritis developed in two patients.

Bronchiectasis and chronic sinusitis were noted in two and six patients, respectively, before the initiation of IVIG replacement. More patients may well have developed these lesions at this early stage, however, no radiologic assessment was available in most cases.

Lymphoid tissue hyperplasia

Lymphoid hyperplasia was noted in 20 patients before IVIG replacement (Table 1). In 14 patients, it could be determined that lymphoid hyperplasia developed at a median age of 6.5 years (range: 0.5 to 28), and the median time to initiation of IVIG therapy was 4.6 years (range: 1.3 to 15). Lymphoid hyperplasia was characterized by hyperplasia of peripheral lymph nodes ($n = 13$), mesenteric lymph nodes ($n = 2$), mediastinal lymph nodes ($n = 1$), spleen ($n = 2$), liver ($n = 2$) and tonsils ($n = 8$). Histological examination of tonsils, spleen and peripheral or mesenteric lymph nodes available in three patients revealed follicular hyperplasia with giant germinal centers as previously reported [26].

Autoimmune and related inflammatory disorders

Autoimmune and related inflammatory disorders developed in six patients (21%) (Table 3). These complications occurred between 3.3 and 8 years of age in four cases, within the second decade of life in one case and in adulthood in one patient. The onset preceded IVIG therapy in five cases (Patients 2, 8, 14, 15 and 20). In Patient 1, marked splenomegaly was present at diagnosis and associated with oral ulcers, thrombocytopenia and a monoclonal IgM kappa peak of 42 g/l; diabetes mellitus requiring daily insulin administration developed at the age of 21 years, shortly after IVIG and corticosteroid therapy had been initiated. This patient also had an episode of cerebral thrombosis at 24 years of age. The case of Patient 2 who developed a chronic, destructive, bilateral and symmetrical polyarthritis with the typical clinical and radiologic features of rheumatoid arthritis has been previously reported [28]. This patient was treated with low-dose prednisone. Another patient (Patient 8) developed autoimmune hepatitis, hemolytic anemia and thrombocytopenia with several autoanti-

Notes to Table 1:
Patients 9, 10 and 11, 12 and 13, 18 and 19, and 27 and 28 are siblings; Patients 4 and 5 and Patients 24 and 25 are cousins. The case of Patient 2 was described in Ref. [28]; Patients 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 16, 18, 19, 20, 22, 23, 24 and 25 were reported in Ref. [26]. Abbreviations: Pt, patient; sympt, symptom; URT = upper respiratory tract; LRT = lower respiratory tract; IVIG, intravenous immunoglobulin; NA, non-available; pneum, pneumonia; *Giardia*, *Giardia lamblia*; fs, frameshift; HSV, Herpes simplex virus; pulm, pulmonary; AIHA, autoimmune hemolytic anemia; ITP, immune thrombocytopenia; AI hepatitis, autoimmune hepatitis; sinus, sinusitis; del, deletion; men, meningitis; ins, insertion.
[a] Previous intramuscular IG substitution for 3.7 years (Patient 23), 6 years (Patients 15 and 27), 8 years (Patient 14) and 10 years (Patient 2), respectively.
[b] With negative rheumatoid factor but typical clinical and radiologic findings as described in a previous publication [28].
[c] Anti-erythrocytes (positive Coomb's test), -platelets, -cardiolipin, -LKM, -liver membrane and -smooth muscle antibodies of IgM isotype.

Table 3
Autoimmune and related inflammatory disorders

Main complications (description)	Patient number[a]	Main associated features
Diabetes mellitus[b]	1	monoclonal IgM kappa, thrombocytopenia, oral ulcers, cerebral thrombosis[c]
Destructive polyarthritis[d]	2	no autoantibody, including RF [26]
AI hepatitis, AIHA and ITP[d]	8[e]	anti-LKM, -smooth muscle, -liver membrane, -platelet, -erythrocyte and -cardiolipin IgM
Crohn's disease[d]	14	negative infection screen, histological diagnosis, response to pentasalazin and corticosteroid therapy
Chronic bilateral uveitis[d]	15	negative infection screen, response to cyclosporin and corticosteroid therapy
Chronic active hepatitis[d]	20	negative infection screen, histological diagnosis

AI, autoimmune; AIHA, autoimmune hemolytic anemia; ITP, Immune thrombocytopenia; RF, rheumatoid factor.
[a] Numbers according to Table 1.
[b] Requiring insulin therapy, diagnosed at 21 years of age, shortly after initiation of IVIG replacement and corticosteroid therapy.
[c] At 24 years of age.
[d] Onset before initiation of IVIG therapy and persistence at the last follow-up.
[e] This patient also experienced hyperviscosity syndrome with transient left ventricular dysfunction, 2 years after initiation of IVIG therapy.

bodies of IgM isotype, including anti-hepatocyte membrane, -liver-kidney-microsome (LKM), -smooth muscle, -cardiolipin,- erythrocyte (Coomb's test) and -platelet antibodies. Treatment consisted of corticosteroids and pulsed IV cyclophosphamide. Chronic hepatitis developed in another patient (Patient 20) and though no autoantibodies were found in this case, its autoimmune origin was supported by a negative infection screen, histological findings and by the efficacy of corticosteroids and immunosuppressive therapy. Patient 14 developed inflammatory bowel disease mimicking Crohn's disease and was treated with pentasalazin and low-dose corticosteroids. Patient 15 had bilateral chronic uveitis, which required corticosteroids and cyclosporin treatment, strongly suggesting an autoimmune disorder although no autoantibody was found.

Treatment and outcome

IVIG therapy was administered to all patients and initiated at a median age of 5.0 years (range: 0.7 to 42 years). Five patients received intramuscular immunoglobulin for 3.7 to 10 years before IVIG therapy was initiated. In the other 24 patients, IVIG replacement was initiated shortly after immunodeficiency was diagnosed. Once initiated, IVIG was administered regularly at least every 4 weeks and serum IgG levels were consistently maintained above 4 or 5 g/l in all but six patients. Low IgG levels were due to poor compliance, were noted in five of the six patients and were secondary to exudative enteropathy with increased IgG losses in the sixth patient. Fifteen patients also received prophylactic antibiotic treatment with oral trimethoprim and sulfamethoxazole. At last evaluation, between July 1999 and July 2003, all patients were alive except for the oldest patient (Patient 2) who died in 2002 at the age of 63 years from septicemia. Patients' median age was 14.2 years (range: 2.7 to 63 years). The median length of follow-up since diagnosis of immunodeficiency was 8.9 years (range: 1.4 to 51). The median length of follow-up from initiation of IVIG was 6.5 years (range: 1.4 to 21.0).

After IVIG therapy was initiated, frequency of infections markedly decreased (Table 2). Despite IVIG therapy, 11 patients still had recurrent URT infections, and five patients had recurrent bronchitis, which had developed in all cases before HIGM2 syndrome was diagnosed. Tonsillectomy and adenoidectomy were reported as effective in five cases in preventing further URT infections. In addition to the patient who died from bacterial sepsis, three patients developed episodes of parenchymal lung infection and another patient septic arthritis. All of these patients, however, had residual serum IgG levels less than 4 g/l when these severe infections developed. Recurrent aseptic arthritis resolved after the initiation of IVIG therapy in two other patients. No opportunistic infections occurred either before or after the onset of IVIG therapy. At last evaluation, chronic sinusitis was present in 14 patients. Of the four patients who developed bronchiectasis, two had bronchiectasis before IVIG treatment and in the other two low IgG levels were recorded.

Lymphoid hyperplasia resolved in 15 of the 20 patients following IVIG therapy but developed anew in two patients (Patients 7 and 12). The seven patients with persistent lymphoid hyperplasia had been on IVIG substitution for a median duration or 8.2 years (ranges 2.2 to 12.7). One patient had episodes of intestinal intussusception probably caused by enlarged mesenteric lymph nodes that developed 2.7 years after the initiation of IVIG and were associated with an exudative enteropathy. These episodes occurred repeatedly over several years and required long-term corticosteroid therapy.

Autoimmune and related inflammatory disorders were well controlled with immunosuppressive therapy in three out of the six patients in whom these complications developed. Of the three poorly controlled patients, one (Patient 8) developed increased liver enzymes during the last few months of follow-up and liver biopsy disclosed chronic active hepatitis which required more intensive immunosuppressive treatment. The other two patients were poorly compliant with treatments and their condition gradually worsened.

Life-threatening complications attributed to hyperviscosity were recorded in one patient (Patient 8) who developed transient left ventricular dysfunction.

Twenty-six of the twenty-eight patients who were alive at last evaluation were working or attending school normally. Of the two remaining patients, one had been diagnosed with

congenital hypothyroidism resulting in impaired neurocognitive development and the other had neurological sequelae and blindness secondary to HSV encephalitis that occurred before HIGM diagnosis.

Immunologic and genetic findings

Serum IgM levels were markedly increased at diagnosis in all cases except in six young children (range 0.71 to 1.6 g/l in these six patients) while IgA and IgG levels were very low or undetectable in all patients (Table 1). Although serum IgM levels decreased in most patients after IVIG therapy was initiated, they frequently remained above normal values. T (CD3+), CD4+, CD8+ and B (CD19+) lymphocyte counts were within normal limits. T cell proliferation tests to phytohemaglutinin A, concanavalin A and recall antigens were also positive in all cases. All B cells expressed normal levels of CD40 molecules and a normal percentage of B cells (20–50%) also expressed CD27. CSR towards IgE as tested in vitro by sCD40-L + IL4 activation was absent in all patients. Isohemagglutinins were detected in 17 out of 19 patients tested for.

Genetic analysis provided evidence of 15 distinct *AID* gene mutations (Table 1); seven missense mutations scattered throughout the gene including the cytidine deaminase domain, 2 mutations generating a premature stop codon, 2 small deletions (9 and 19 bp, the last one leading to a premature stop codon), one mutation of the fifth exon splice acceptor site leading to an RNA transcript 93 bp longer than normal and a deletion of the entire gene region confirmed by Southern blot analysis in five patients from three unrelated families. Homozygous mutations were most often observed (24 patients in 20 families) while heterozygous mutations were detected in five patients from three different families. Same mutations were found in families from related ethnic origin (Moroccove, Sepharadic Jewish or Turkish) and could be related to a common ancestor, as suggested by polymorphic marker analysis [25]. When patients sharing the same mutation were compared for their clinical and immunologic phenotype, some similarities were observed. As an example, Crohn's disease and chronic uveitis, two possibly related inflammatory conditions, occurred in two patients (Patients 14 and 15) who had the same mutation but were not related. However, there were discrepancies in age at disease onset, occurrence of severe infections, intensity of lymphoid hyperplasia, autoimmune and/or inflammatory complications and serum IgM levels between most cases sharing the same mutation.

Discussion

We have described clinical, immunologic and genetic characteristics of 29 patients with autosomal recessive hyper-IgM syndrome caused by *AID* gene mutations (HIGM2 syndrome). The spectrum of clinical manifestations associated with AID deficiency was found wider in this series than it has previously been reported [20]. While infections and lymphoid hyperplasia were common complications, as previously reported, diverse chronic autoimmune and inflammatory manifestations developed in 21% of patients. In addition, a wide array of *AID* gene mutations characterized the present series.

Severe and recurrent infections were the most common features, as previously described [20]. Recurrent URT infections or bronchitis, which were recorded before the age of 10 years in most cases, probably developed early in life in all patients. After IVIG replacement was initiated, the frequency of infections was markedly reduced. However, chronic inflammatory sinus and bronchial lesions had developed in several patients at last follow-up. Late initiation of, or insufficient IVIG therapy, was probably associated with the higher risk of developing such lesions. It is striking to note that patients with HIGM2 were not found to be susceptible to enteroviral infections as observed in agammaglobulinemic patients [30,31]. This suggests that the presence of IgM antibodies, even in the absence of somatic hypermutations, represent an effective means of defense, at least against some infectious agents. It would be interesting in the future to compare susceptibility to infections of AID-deficient patients with that of HIGM4 patients whose B cells exhibit normal SHM [23]. The absence of detectable opportunistic infection in patients with HIGM2, as previously reported, is in accordance with a pure B-cell deficiency. The fact that one patient had HSV encephalitis may be fortuitous, as severe HSV infections can occur in patients without immunodeficiency. Associated deficiency of T cells or cells of the monocyte–macrophage lineage is unlikely given the pattern of expression of AID, which is strictly restricted to activated B cells [25]. A role for B cells and the secondary antibody response in the prevention of these infections are also conceivable [32].

Lymphoid hyperplasia, which developed in 69% of patients, has previously been reported as a common feature in patients with AID deficiency [20,26,33]. The intense proliferation of germinal center B cells which leads to giant germinal centers is likely not due to a defect in apoptosis since *fas* molecule is normally expressed and numerous macrophages containing apoptotic inclusions are normally observed [26]. One possible explanation for the intense proliferation observed is that, in the absence of functional AID, antigens continuously induce B-cell proliferation [34]. Alternatively, AID may exert direct control over GC B-cell proliferation. This intense B-cell proliferation may theoretically result in an increased risk of lymphoma, however, this complication has not been so far reported in AID-deficient patients. Interestingly, IVIG therapy was associated with resolution of lymphoid hyperplasia in 75% of the cases (15 out of 20) while only two patients developed lymphoid hyperplasia following IVIG administration. Polyvalent immunoglobulins G could simply act through their ability to control and/or prevent recurrent or chronic infections

although other immunomodulatory mechanisms through Fcγ receptors can be envisaged. Whatever the mechanisms, this observation reinforces the principle that IVIG therapy should be initiated early in AID deficient patients.

Autoimmunity and related inflammatory disorders represented the third most common complication in our series. Although (multiple) autoantibodies of IgM isotype were only found in one patient, there were strong clinical and either imaging or histological evidence for the autoimmune nature of the disorder in the patients with chronic destructive polyarthritis, Crohn's disease, uveitis or chronic active hepatitis. We cannot formally exclude a role for unidentified microorganisms in these patients as well as in the patient who developed an exudative enteropathy. Nevertheless, infection screens were negative in all. In addition, although IVIG therapy was not effective in these patients, corticosteroids and immunosuppressive drugs were. Potentially unselected IgM, which have not undergone SHM, may play a role in the pathogenesis of these autoimmune manifestations. Type 1 diabetes mellitus, which occurred in one patient, could be considered an autoimmune feature as it developed early in life (though corticosteroid therapy was likely a cofactor), but it cannot be ascertained that it was related to HIGM2 deficiency. Recurrent aseptic arthritis, which occurred in two patients, may have been of infectious origin, as observed in patients with agammaglobulinemia [35].

Contrasting with the small number of *AID* gene mutations found in a series of 18 patients with HIGM2 syndrome described by Minegishi et al. [20] and in a recent small series of patients of Japanese origin [33], we found 15 distinct genetic alterations (from missense mutations to complete deletion of the *AID* gene) which all led to the same HIGM2 phenotype. This observation provides evidence for a strong structural constraint of AID which is an evolutionary conserved gene between mice [25] and humans [36]. The small number of patients carrying the same mutation precludes the establishment of a statistically significant genotype/phenotype correlation. The phenotypic homogeneity of patients sharing the same mutation is not absolute and requires further assessment in a larger number of patients to detect potential modifier elements. Minegishi et al. [20] reported on the occurrence of one or more episodes of bacterial meningitis in several French Canadian patients who had the same *AID* gene mutation. We cannot discount the possibility that this was related to additional susceptibility elements either inherited or environmental.

In conclusion, AID deficiency is associated with an increased risk of severe infections, recurrent infections leading to chronic sinusitis and bronchiectasis, lymphoid hyperplasia and also in some patients with autoimmune and inflammatory disorders. Early-onset IVIG therapy is required to prevent infectious complications. The relative contribution of AID mutation types and other genetic or environmental factors has still to be investigated in a larger cohort of patients.

Acknowledgments

We thank Drs. A. Huissoon, Necil Kütükçüler and Nuran Salman for their participation to this study. We thank Dr. Daniel Turnberg for critical reading and corrections. We acknowledge Mrs. M. Forveille for excellent technical assistance.

This work was supported by INSERM (N. Catalan, A. Fischer, A. Durandy), CEE contract no. QLG1-CT-2001-01536- IMPAD (S. Plebani, A. Durandy), l'Association de la Recherche contre le Cancer, la Ligue contre le cancer and the Louis Jeantet Fondation.

References

[1] L.D. Notarangelo, M. Duse, A.G. Ugazio, Immunodeficiency with hyper-IgM (HIM), Immunodefic. Rev. 3 (1992) 101–121.
[2] R.C. Allen, R.J. Armitage, M.E. Conley, H. Rosenblatt, N.A. Jenkins, N.G. Copeland, M.A. Bedell, S. Edelhoff, C.M. Disteche, D.K. Simoneaux, et al., CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome, Science 259 (1993) 990–993.
[3] A. Aruffo, M. Farrington, D. Hollenbaugh, X. Li, A. Milatovich, S. Nonoyama, J. Bajorath, L.S. Grosmaire, R. Stenkamp, M. Neubauer, et al., The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome, Cell 72 (1993) 291–300.
[4] J.P. DiSanto, J.Y. Bonnefoy, J.F. Gauchat, A. Fischer, G. de Saint Basile, CD40 ligand mutations in x-linked immunodeficiency with hyper-IgM, Nature 361 (1993) 541–543.
[5] L.D. Notarangelo, M.C. Peitsch, CD40lbase: a database of CD40L gene mutations causing X-linked hyper-IgM syndrome, Immunol. Today 17 (1996) 511–516.
[6] F. Rousset, E. Garcia, J. Banchereau, Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen, J. Exp. Med. 173 (1991) 705–710.
[7] K. Zhang, E.A. Clark, A. Saxon, CD40 stimulation provides an IFN-gamma-independent and IL-4-dependent differentiation signal directly to human B cells for IgE production, J. Immunol. 146 (1991) 1836–1842.
[8] F.M. Uckun, K. Gajl-Peczalska, D.E. Myers, W. Jaszcz, S. Haissig, J.A. Ledbetter, Temporal association of CD40 antigen expression with discrete stages of human B-cell ontogeny and the efficacy of anti-CD40 immunotoxins against clonogenic B-lineage acute lymphoblastic leukemia as well as B-lineage non-Hodgkin's lymphoma cells, Blood 76 (1990) 2449–2456.
[9] F.M. Uckun, G.L. Schieven, I. Dibirdik, M. Chandan-Langlie, L. Tuel-Ahlgren, J.A. Ledbetter, Stimulation of protein tyrosine phosphorylation, phosphoinositide turnover, and multiple previously unidentified serine/threonine-specific protein kinases by the Pan-B-cell receptor CD40/Bp50 at discrete developmental stages of human B-cell ontogeny, J. Biol. Chem. 266 (1991) 17478–17485.
[10] J. Levy, T. Espanol-Boren, C. Thomas, A. Fischer, P. Tovo, P. Bordigoni, I. Resnick, A. Fasth, M. Baer, L. Gomez, E.A. Sanders, M.D. Tabone, D. Plantaz, A. Etzioni, V. Monafo, M. Abinun, L. Hammarstrom, T. Abrabamsen, A. Jones, A. Finn, T. Klemola, E. DeVries, O. Sanal, M.C. Peitsch, L.D. Notarangelo, Clinical spectrum of X-linked hyper-IgM syndrome, J. Pediatr. 131 (1997) 47–54.
[11] P. Dent, Spectrum of the X-linked hyper IgM syndrome, J. Pediatr. 132 (1998) 561–562.
[12] C.K. Cunningham, C.A. Bonville, H.D. Ochs, K. Seyama, P.A. John, H.A. Rotbart, L.B. Weiner, Enteroviral meningoencephalitis as a complication of X-linked hyper IgM syndrome, J. Pediatr. 134 (1999) 584–588.

[13] L.D. Notarangelo, A.R. Hayward, X-linked immunodeficiency with hyper-IgM (XHIM), Clin. Exp. Immunol. 120 (2000) 399–405.
[14] R. Doffinger, A. Smahi, C. Bessia, F. Geissmann, J. Feinberg, A. Durandy, C. Bodemer, S. Kenwrick, S. Dupuis-Girod, S. Blanche, P. Wood, S.H. Rabia, D.J. Headon, P.A. Overbeek, F. Le Deist, S.M. Holland, K. Belani, D.S. Kumararatne, A. Fischer, R. Shapiro, M.E. Conley, E. Reimund, H. Kalhoff, M. Abinun, A. Munnich, A. Israel, G. Courtois, J.L. Casanova, X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling, Nat. Genet. 27 (2001) 277–285.
[15] A. Jain, C.A. Ma, S. Liu, M. Brown, J. Cohen, W. Strober, Specific missense mutations in NEMO result in hyper-IgM syndrome with hypohydrotic ectodermal dysplasia, Nat. Immun. 2 (2001) 223–228.
[16] S. Mansour, H. Woffendin, S. Mitton, I. Jeffery, T. Jakins, S. Kenwrick, V.A. Murday, Incontinentia pigmenti in a surviving male is accompanied by hypohydrotic ectodermal dysplasia and recurrent infection, Am. J. Med. Genet. 99 (2001) 172–177.
[17] M.E. Conley, M. Larche, V.R. Bonagura, A.R. Lawton III, R.H. Buckley, S.M. Fu, E. Coustan-Smith, H.G. Herrod, D. Campana, Hyper IgM syndrome associated with defective CD40-mediated B cell activation, J. Clin. Invest. 94 (1994) 1404–1409.
[18] R.E. Callard, S.H. Smith, J. Herbert, G. Morgan, M. Padayachee, S. Lederman, L. Chess, R.A. Kroczek, W.C. Fanslow, R.J. Armitage, CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM). Comparison of X-linked, autosomal recessive, and non-X-linked forms of the disease, and obligate carriers, J. Immunol. 153 (1994) 3295–3306.
[19] A. Durandy, C. Hivroz, F. Mazerolles, C. Schiff, F. Bernard, E. Jouanguy, P. Revy, J.P. DiSanto, J.F. Gauchat, J.Y. Bonnefoy, J.L. Casanova, A. Fischer, Abnormal CD40-mediated activation pathway in B lymphocytes from patients with hyper-IgM syndrome and normal CD40 ligand expression, J. Immunol. 158 (1997) 2576–2584.
[20] Y. Minegishi, A. Lavoie, C. Cunningham-Rundles, P.M. Bedard, J. Hebert, L. Cote, K. Dan, D. Sedlak, R.H. Buckley, A. Fischer, A. Durandy, M.E. Conley, Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome, Clin. Immunol. 97 (2000) 203–210.
[21] S. Ferrari, S. Giliani, A. Insalaco, A. Al-Ghonaium, A.R. Soresina, M. Loubser, M.A. Avanzini, M. Marconi, R. Badolato, A.G. Ugazio, Y. Levy, N. Catalan, A. Durandy, A. Tbakhi, L.D. Notarangelo, A. Plebani, Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper IgM, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 12614–12619.
[22] N. Kutukculer, D. Moratto, Y. Aydinok, V. Lougaris, S. Aksoylar, A. Plebani, F. Genel, L.D. Notarangelo, Disseminated *Cryptosporidium* infection in an infant with hyper-IgM syndrome caused by CD40 deficiency, J. Pediatr. 142 (2003) 194–196.
[23] K. Imai, N. Catalan, A. Plebani, L. Marodi, O. Sanal, S. Kumaki, V. Nagendran, P. Wood, C. Glastre, F. Sarrot-Reynauld, O. Hermine, M. Forveille, P. Revy, A. Fischer, A. Durandy, Hyper-IgM syndrome type 4 with a B lymphocyte-intrinsic selective deficiency in Ig class-switch recombination, J. Clin. Invest. 112 (2003) 136–142.
[24] K. Imai, G. Slupphaug, W.I. Lee, P. Revy, S. Nonoyama, N. Catalan, L. Yel, M. Forveille, B. Kavli, H.E. Krokan, H.D. Ochs, A. Fischer, A. Durandy, Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination, Nat. Immunol. 4 (2003 Oct) 1023–1028.
[25] M. Muramatsu, V.S. Sankaranand, S. Anant, M. Sugai, K. Kinoshita, N.O. Davidson, T. Honjo, Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells, J. Biol. Chem. 274 (1999) 18470–18476.
[26] P. Revy, T. Muto, Y. Levy, F. Geissmann, A. Plebani, O. Sanal, N. Catalan, M. Forveille, R. Dufourcq-Labelouse, A. Gennery, I. Tezcan, F. Ersoy, H. Kayserili, A.G. Ugazio, N. Brousse, M. Muramatsu, L.D. Notarangelo, K. Kinoshita, T. Honjo, A. Fischer, A. Durandy, Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2), Cell 102 (2000) 565–575.
[27] M. Muramatsu, K. Kinoshita, S. Fagarasan, S. Yamada, Y. Shinkai, T. Honjo, Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme, Cell 102 (2000) 553–563.
[28] J. Sibilia, A. Durandy, T. Schaeverbeke, J.P. Fermand, Hyper-IgM syndrome associated with rheumatoid arthritis: report of RA in a patient with primary impaired CD40 pathway, Br. J. Rheumatol. 35 (1996) 282–284.
[29] U. Klein, K. Rajewsky, R. Kuppers, Human immunoglobulin (Ig)M+IgD+ peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells, J. Exp. Med. 188 (1998) 1679–1689.
[30] P.J. Mease, H.D. Ochs, L. Corey, J. Dragavon, R.J. Wedgwood, Echovirus encephalitis/myositis in X-linked agammaglobulinemia, N. Engl. J. Med. 313 (1985) 758.
[31] R.E. McKinney Jr., S.L. Katz, C.M. Wilfert, Chronic enteroviral meningoencephalitis in agammaglobulinemic patients, Rev. Infect. Dis. 9 (1987) 334–356.
[32] M. Bystricka, M. Petrikova, M. Zatovicova, L. Solarikova, F. Kostolansky, V. Mucha, G. Russ, Monoclonal antibodies to the distinct antigenic sites on glycoproteins C and B and their protective abilities in herpes simplex virus infection, Acta Virol. 41 (1997) 5–12.
[33] Y. Zhu, S. Nonoyama, T. Morio, M. Muramatsu, T. Honjo, S. Mizutani, Type two hyper-IgM syndrome caused by mutation in activation-induced cytidine deaminase, J. Med. Dent. Sci. 50 (2003) 41–46.
[34] S. Faragasan, K. Kinoshita, M. Muramatsu, K. Ikuta, T. Honjo, In situ class switching and differentiation to IgA-producing cells in the gut lamina propria, Nature 413 (2001) 639–643.
[35] H.D. Ochs, C.I. Smith, X-linked agammaglobulinemia. A clinical and molecular analysis, Medicine (Baltimore) 75 (1996) 287–299.
[36] T. Muto, M. Muramatsu, M. Taniwaki, K. Kinoshita, T. Honjo, Isolation, tissue distribution, and chromosomal localization of the human activation-induced cytidine deaminase (AID) gene, Genomics 68 (2000) 85–88.