

Inducible CO-stimulator molecule, a candidate gene for defective isotype switching, is normal in patients with hyper-IgM syndrome of unknown molecular diagnosis

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Background: Inducible CO-stimulatory molecule (ICOS), the third member of the CD28/CTLA4 family, is expressed by activated T cells and interacts with its ligand (ICOSL, B7-related protein-1 [B7RP-1]) that is constitutively expressed by B cells. The interaction of ICOS with its ligand leads to terminal differentiation of B cells to plasma cells. ICOS-deficient mice fail to undergo immunoglobulin-class switch recombination (CSR) and germinal center formation, suggesting that ICOS could be a candidate gene for phenotypic hyper-IgM (HIGM) syndromes characterized by recurrent infections, low serum IgG and IgA, and normal or elevated IgM. Genetically, at least 4 distinct molecular defects have been identified that result in defective CSR and present as HIGM syndromes: defects of the CD40 ligand gene (CD40L; HIGM1, X-linked), the activation-induced cytidine deaminase gene (AID; HIGM2, autosomal recessive), the CD40 gene (HIGM3, autosomal recessive), and the nuclear factor- κ B (NF- κ B) essential modulator gene (NEMO, or IKK- γ , X-linked). In a substantial subgroup of HIGM patients, these 4 genes are normal, and the genetic defect is unknown.

Objective: We sought to investigate the possibility that mutations of ICOS could account for some patients' belonging to this HIGM subgroup.

Methods: The expression of ICOS protein by activated peripheral blood mononuclear cells and/or interleukin-2-dependent T cell lines derived from these patients was estimated by flow cytometry after incubation of the cells with the ICOS ligand fusion protein B7RP-1 Fc. The coding region and exon-intron boundaries of ICOS were assessed by sequence analysis.

Results: We studied 33 HIGM patients from 30 families, selected from an original cohort of 136 patients from 113 families, by excluding mutations of CD40L, AID, CD40, and NEMO.

Activated T cells from all 33 patients expressed ICOS normal-

ly, and sequence analysis of the coding region and exon-intron boundaries revealed only wild-type ICOS, predicting that the protein structure is normal in this patient population.

Conclusion: These findings strongly suggest that ICOS does not belong to the group of genes that, if mutated, present clinically as the HIGM syndrome. (*J Allergy Clin Immunol* 2003;112:958-64.)

Key words: Hyper-IgM phenotypic immunodeficiency disorders; hyper IgM syndrome, inducible costimulatory molecule, common variable immunodeficiency

Patients with the hyper-IgM syndrome (HIGM) present with low serum levels of IgG and IgA and normal or elevated IgM, defective isotype switching (class switch recombination [CSR]), and recurrent, severe infections.^{1,2} Four distinct molecular defects causing HIGM have been identified. The first gene discovered, *CD40 ligand* (*CD40L*, *CD154*), is expressed by activated CD4⁺ lymphocytes and, if mutated, is responsible for X-linked HIGM (XHIGM, HIGM1).³⁻⁷ Subsequently, mutations of the *activation-induced cytidine deaminase* (*AID*) gene were discovered in a subgroup of HIGM patients (HIGM2), who often present with lymph node and tonsillar hyperplasia but without opportunistic infections.⁸ Because CD40-defective mice have a characteristic antibody deficiency, including absent CSR, and abnormal germinal center formation,^{9,10} it was not unexpected that 2 families with an autosomal form of HIGM were found to have mutations of the *CD40* gene (HIGM3)¹¹; CD40-deficient patients are susceptible to opportunistic infections, similar to patients with mutations of *CD40L*. Recently, male HIGM patients with ectodermal dysplasia were identified to have characteristic mutations of the *nuclear factor- κ B* (*NF- κ B*) essential modulator gene (*NEMO*, also known as IKK- γ), thus impairing NF- κ B signaling.¹²⁻¹⁴ In an effort to identify the molecular basis of HIGM, we and others (A. Durandry, Paris, personal communication) have accumulated information on HIGM patients with normal *CD40L*, *AID*, *CD40*, and *NEMO* genes.

In this article, we examine the possibility that mutations of the *inducible costimulator molecule* gene (*ICOS*, located at 2q33) might be responsible for a subset of patients with this molecularly undefined form of HIGM syndrome. ICOS, first described in 1999 by Hutloff and

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Abbreviations used:

AID:	Activation-induced cytidine deaminase
B7RP-1:	B7-related protein-1
CSR:	Class switch recombination
CVID:	Common variable immunodeficiency
HIGM:	Hyper-IgM phenotypic immunodeficiency disorders, Hyper-IgM syndrome
ICOS:	Inducible costimulatory molecule
IL:	Interleukin
NEMO:	Nuclear factor- κ B (NF- κ B) essential modulator
PBMC:	Peripheral blood mononuclear cell
RT-PCR:	Reverse transcriptase-polymerase chain reaction
TCR:	T-cell receptor

coworkers,¹⁵ is the third member of the CD28/CTLA-4 family. The ICOS gene is clustered on chromosome 2q33, together with *CD28* and *CTLA4*, and consists of 5 exons and 4 introns.^{15,16} Human ICOS, a homodimer with 39% similarity to both CD28 and CTLA-4 at the protein level, is de novo expressed on the surface of activated T cells. Unstimulated T cells or T cells preactivated only through the T-cell receptor (TCR) are unable to induce B cells to synthesize immunoglobulins. After the initial activation of T cells through the TCR, CD40L is expressed and interacts with B cells via CD40. As a direct result, B cells upregulate B7, which interacts with CD28, thereby inducing expression of ICOS on the surfaces of T cells. These events result in the upregulation of CTLA-4, which terminates the interaction of B7 and CD28. In a second round of cell-cell cross-talk, ICOS is expected to continue to initiate costimulatory events, resulting in increased cytokine secretion by T cells, thus supporting the terminal differentiation of B cells and immunoglobulin secretion.¹⁵ The interaction of ICOS and B7-related protein-1 (B7RP-1), the natural ligand of ICOS, constitutively expressed by B cells, monocytes, and activated fibroblasts,^{17,18} effectively induces interleukin (IL)-10 secretion.^{15,16,19} In germinal centers, ICOS-B7RP-1 signaling and the resulting IL-10 generation are expected to lead to terminal differentiation of B cells into plasma cells in the apical part of the light zone, which is the major site of ICOS expression.¹⁶ For these reasons and the observation that ICOS-deficient mice have impaired germinal center formation and defective immunoglobulin class switching,²⁰ we hypothesized that ICOS might play a critical role in B-cell activation and isotype switching and therefore, could be a candidate gene for a subgroup of HIGM patients who were shown to have normal genes known to cause HIGM when mutated.

METHODS

Patients

Patients with low serum IgG and IgA (≤ 2 SD below the normal values for age), normal or elevated IgM, and a history of recurrent or episodic infections were enrolled in the study, regardless of sex, age, and family history. Patients with human immunodeficiency virus

infection, a history of congenital rubella, antiepileptic drug exposure, and other known immunodeficiency disorders or neoplasia were excluded. After informed consent was obtained, 10 to 20 mL of heparinized, venous blood was obtained from each patient and, when indicated, from a normal control subject. Primary lymphocytes and/or IL-2-dependent T-cell lines were analyzed, and only HIGM patients with normal CD40 ligand, CD40, AID, and NEMO (if any clinical signs of hypohydrotic ectodermal dysplasia or incontinentia pigmenti were present) were accepted into the study.

Cell preparation and culture

Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by Ficoll-Hypaque (Pharmacia Biotech, Piscataway, NJ) centrifugation. RPMI-1640 supplemented with 10% fetal calf serum (Hyclone, Logan, Utah), 2 mmol/L glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin (complete medium) was used for cell culture. IL-2-dependent T-cell lines were established from primary lymphocytes by a standard method.²¹ In brief, we added human 500 U/mL recombinant IL-2 (Leinco, St Louis, Mo) and 1 μ g/mL phytohemagglutinin (Sigma, St Louis, Mo) to the complete medium and changed the culture medium containing IL-2/PHA every 4 days. After 3 weeks in culture, the T-cell numbers had increased by 8-fold (approximately 4×10^7), and the cells were studied for ICOS expression; the remaining cells were frozen in liquid nitrogen.

ICOS expression by activated T cells

Lymphocytes were activated and ICOS was identified by immunostaining, as described previously.^{15,18} PBMCs were first stained with phycoerythrin-labeled anti-CD4 monoclonal antibody (mouse IgG1; Ancell, Bayport, Minn) at 4°C for 20 minutes, followed by stimulation with phorbol 12-myristate 13-acetate (PMA) (GIBCO-BRL, Gaithersburg, Md) at 10 ng/mL and ionomycin at 1 μ g/mL (Sigma, St Louis, Mo) for 2 to 24 hours. Immunostaining for expressed ICOS was performed by incubating the activated cells with the ICOS ligand fusion protein B7RP-1 Fc (a gift from Amgen Inc) at a concentration of 20 μ g/mL for 1 hour at room temperature; the cells were then washed and stained with fluorescein isothiocyanate-conjugated anti-human Fc antibody (Chemicon, Temecula, Calif) at a final dilution of 1:200. In parallel experiments, we measured the activation molecule CD69 with a fluorescein isothiocyanate-labeled anti-CD69 monoclonal antibody (Becton Dickinson, San Jose, Calif). The stained cells were analyzed by FACScan (Becton Dickinson, San Jose, Calif); a minimum of 10,000 events was collected, and the results were analyzed by Lysis II software (Becton Dickinson).

Sequence analysis of the ICOS gene

Total RNA was isolated from activated PBMCs cultured for 6 to 8 hours in the presence of PMA at 10 ng/mL and ionomycin at 1 μ g/mL or from IL-2-dependent T-cell lines with TRIzol (Life Tech, Carlsbad, Calif). Reverse transcription (RT) of mRNA followed by polymerase chain reaction (PCR) was performed as previously described.²¹ In brief, 1 μ g RNA in a total volume of 20 μ L was RT into cDNA with the use of oligo-dT primers (Invitrogen, Carlsbad, Calif) and superscript RNaseH⁻ reverse transcriptase (Qiagen, Chatsworth, Calif). Amplification was performed with 1 μ L of cDNA in a total volume of 20 μ L containing 0.5 U high-fidelity *Taq* DNA polymerase (Invitrogen), 1.875 mmol/L $MgSO_4$, 200 μ mol/L dNTP, 500 nmol/L oligonucleotide forward primer (5' $\rightarrow$ 3') GAA CAC TGA ACG CGA GGA CT and reverse primer (5' $\rightarrow$ 3') GTT GAG GAA AAC TGG CCA AC, and 10 $\times$ buffer (Invitrogen). The oligonucleotide primers selected covered the entire coding region of the ICOS gene. Genomic DNA from a representative group of 17

TABLE I. Clinical features of HIGM patients (n = 33) with normal *CD40L*, *AID*, *CD40*, and *NEMO**

Sex	
Male	24
Female	9
Age of onset (y)	
<1	8
1-3	13
4-10	8
>10	4
Clinical presentation	
Recurrent pneumonia	29 (88%)
Recurrent otitis	21 (64%)
Recurrent sinusitis	19 (58%)
Lymphoid hyperplasia	11 (33%)
Hepatosplenomegaly	8 (24%)
CNS disorders	7 (21%)
Failure to thrive	5 (15%)
Bronchiectasis	5 (15%)
Chronic diarrhea	5 (15%)
<i>Pneumocystis carinii</i> pneumonia	4 (12%)
Mortality	4 (12%)

CNS, Central nervous system.

NEMO* gene was sequenced in 5 patients with findings resembling hypohydrotic ectodermal dysplasia.TABLE II.** Laboratory findings

Serum immunoglobulin level*	Low	Normal	High
IgG	25	7‡	1‡
IgA	32	—	1§
IgM	—	16	17
Absolute lymphocyte count (29)†	12 (41%)	12 (41%)	5 (18%)
CD3 (27)	12 (44%)	11 (41%)	4 (15%)
CD19 (22)	11 (50%)	8 (36%)	3 (14%)
NK (17)	14 (82%)	3 (18%)	—
CD4/CD8 ratio (28)	9 (32%)	16 (57%)	3 (11%)
T-cell proliferation†			
Mitogen (20)#	4 (20%)	16 (80%)	—
Antigen (15)	7 (47%)	8 (53%)	—

*Normal ranges from: Stiehm RE. Immunologic disorders in infants and children. 5th ed. Philadelphia: Philadelphia Press; 1998.

†Number of patients in whom the test was performed.

‡IgG levels were assessed while on intravenous immunoglobulin therapy

§One of 3 affected members from the same family; 2 had low IgG and IgA and normal or elevated IgM.

#Mitogens (PHA, PMA, Con A).

||Antigens (tetanus, *Candida*, or diphtheria).

patients was isolated from whole blood, and amplification of each of the 5 exons, including the exon-intron boundaries, was performed with the following primers pairs: E1S (5' → 3') GAA ACA CCC CCT TGA TTT GA; E1AS (5' → 3') CAC TGT CTT TTG GGT GCG TA; E2S (5' → 3') GCT GTT TCA TTT TGG TGC AA; E2AS (5' → 3') GCA TCT AAG TGA ACT CCA ACA CA; E3S (5' → 3') TGC CCA CTT ACT GGA TTT CA; E3AS (5' → 3') TTG TCT CCC TGT TGG TCA AA; E4S (5' → 3') GCC TAA AGC CAG GAA CCT AA; E4AS (5' → 3') GGC CTT GGA GAT GTT TTC CT; E5S (5' → 3') TAT GAA AGG CAA TGG AGA GG; and E5AS (5' → 3') TTG AAC AAA ACA CCA GCA GAA. Sequencing of the products was performed with the Big Dye Terminator kit (Applied Biosystems, Foster City, Calif) and an ABI PRISM 3100 DNA Sequencer (Applied Biosystems).

RESULTS

Patients

Between 1992 and 2002, we established IL-2-dependent T-cell lines and/or isolated RNA from 136 patients from 113 unrelated families with the HIGM phenotype. After excluding mutations of *CD40L*, *AID*, and *CD40* (and *NEMO* in those with hypohydrotic ectodermal dysplasia and/or incontinentia pigmenti), 33 patients (24 males, 9 females) from 30 families (23 white, 2 African-American, 2 Asian, 1 Native American, 1 of Middle Eastern descent, and 1 Hispanic) were analyzed for mutations of *ICOS*. All patients included in the study presented with recurrent infections, the most frequent being pneumonia, otitis media, and sinusitis (Table I). Opportunistic infections were rare; 4 patients (12%) had a history of *Pneumocystis carinii* pneumonia. Onset of symptoms at ≤10 years of age was observed in 29 of 33 patients. Seventeen patients (52%) had elevated serum IgM levels at the time of diagnosis (Table II). IgG was low for age in 25 patients and not evaluable for the remaining 8 patients who were receiving intravenous immunoglobulin therapy when evaluated; IgA was absent or low in 32 patients. Two of 29 patients, both <1 year of age, were lymphopenic (456/mm³ and 827/mm³, respectively). Abnormal in vitro lymphocyte proliferation to mitogens and to specific antigens was observed in 4 of 20 and 7 of 15 patients, respectively. A reverse CD4/CD8 ratio was observed in 9 of 28 patients studied. Hepatosplenomegaly was present in 8 patients (24%), and enlarged lymph nodes were noted in 11 patients (33%). Massive mediastinal lymph node hyperplasia developed in 2 patients, causing Horner syndrome and obstructive heart failure. Four patients subsequently died, 2 of malignancy (colon carcinoma, Hodgkin's lymphoma) and 2 of sepsis.

ICOS expression by activated CD4⁺ T cells

Unstimulated PBMCs from normal control subjects and HIGM patients did not bind B7RP-1, the natural ligand of ICOS. Expression of ICOS by CD4⁺ cells was detectable 2 hours after stimulation with PMA and ionomycin. After 24 hours of stimulation, 80% to 90% of CD4⁺ cells strongly expressed ICOS, as shown by the binding of B7RP-1 fusion protein (Fig 1, A). Normal IL-2-dependent T-lymphoblastoid cells constitutively expressed ICOS at maximal intensity. Further activation with PMA/ionomycin increased CD69 expression but had no additional effect on the intensity of ICOS expression (Fig 1, B). PBMCs from 2 HIGM patients (representative patient B.C.) and IL-2-dependent T-cell lines from the remaining 31 patients (representative patient W.J.) were surveyed for ICOS expression with the same procedure: 97% of activated CD4⁺ T cells (B.C.) and 99% of unstimulated IL-2-dependent T lymphoblasts (W.J.) expressed ICOS (Fig 1, C and D). In every instance, ICOS expression by patient cells was similar in intensity and kinetics to that of control subjects.

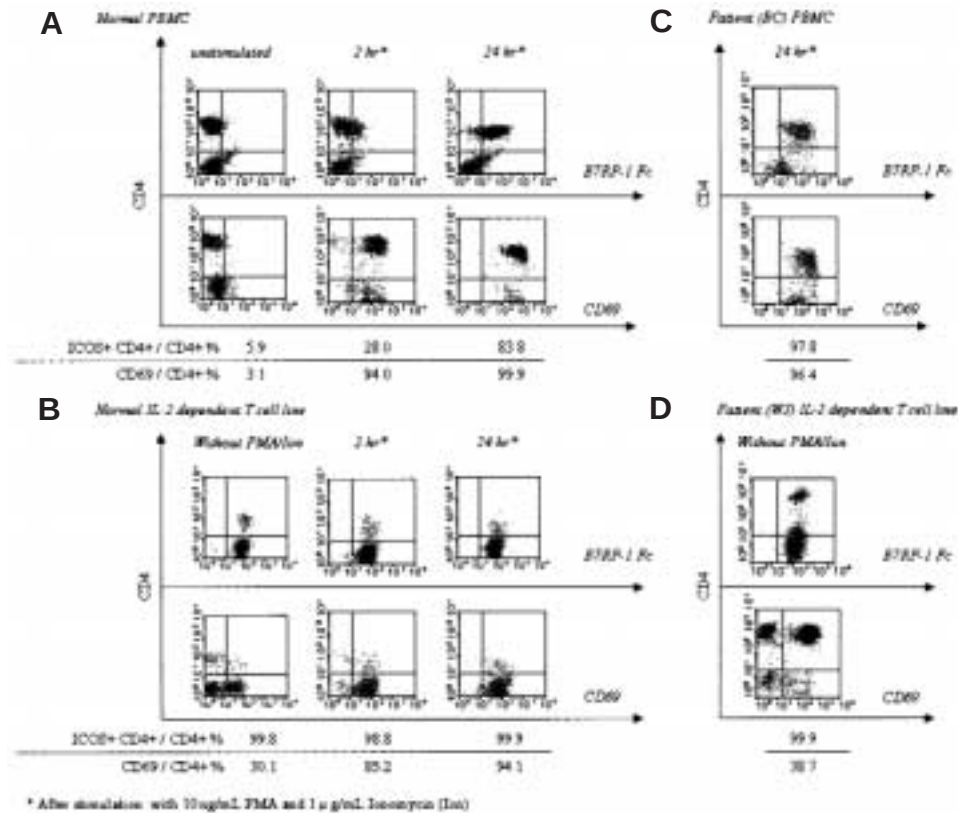


FIG 1. ICOS protein expression by activated CD4⁺ cells and IL-2-dependent T lymphoblastoid cells. Normal (A) and representative patient BC (C) PBMCs were stimulated with PMA and Ionomycin for the time indicated. ICOS expression by CD4⁺ T cells was determined by flow cytometry through use of the ICOS ligand fusion protein (B7RP-1 Fc). CD69 served as activation control. ICOS expression was observed only in activated CD4⁺ cells and equally well by patient BC. IL-2-dependent T-cell lines from a normal control (B) and a representative patient, WJ (D), expressed ICOS maximally; additional stimulation did not increase ICOS expression.

ICOS transcription and sequence analysis

By RT-PCR, transcription of ICOS could not be detected in unstimulated control and patient PBMCs, even after 30 cycles of amplification. Transcription of ICOS could be detected in PBMCs after 2 hours of activation and had increased in quantity after 6 and 24 hours of activation. IL-2-dependent T-cell lines expressed ICOS message constitutively and increased ICOS expression in response to additional activation; product could be detected after 15 cycles of amplification (Fig 2, A), as expected from flow cytometry data. RT-PCR of mRNA isolated from activated PBMCs (18 HIGM patients) or from IL-2-dependent T-cell lines (15 HIGM patients) showed a pattern identical to that of normal control subjects both in quantity and size (Fig 2, B and C). Sequence analysis of ICOS cDNA was normal in all 33 patients analyzed, predicting wild-type ICOS protein. We identified 1 polymorphism (A → C) at nucleotide 669 (Gene Bank AJ 277832), which is part of the first codon after the stop codon (Fig 3). The affinity of ICOS to B7RP-1 Fc was not different between homozygous (669A/A, 669C/C) and heterozygous (669A/C) alleles

(data not shown). Analysis of genomic DNA isolated from 17 patients showed normal sequences for each exon, including the exon-intron boundaries.

DISCUSSION

The clinical phenotype of HIGM represents a heterogeneous group of patients with immunodeficiency, hypogammaglobulinemia, and normal or elevated IgM. Family studies suggest X-linked or autosomal recessive inheritance, but sporadic cases are common. To date, 4 genes, when mutated, have been recognized to cause HIGM. The products of these genes have in common the characteristic that they directly or indirectly affect B-cell signaling or isotype switching and somatic hypermutation. Patients with mutations of either CD40 ligand (X-linked) or CD40 (autosomal recessive) present early in life with severe infections, including opportunistic infections such as *Pneumocystis carinii* pneumonia. In contrast, patients with mutations of AID have a milder clinical phenotype, with delayed onset and without increased risk of opportunistic infections. Patients with mutations of NEMO (X-linked) present with hypohydrotic ectoder-

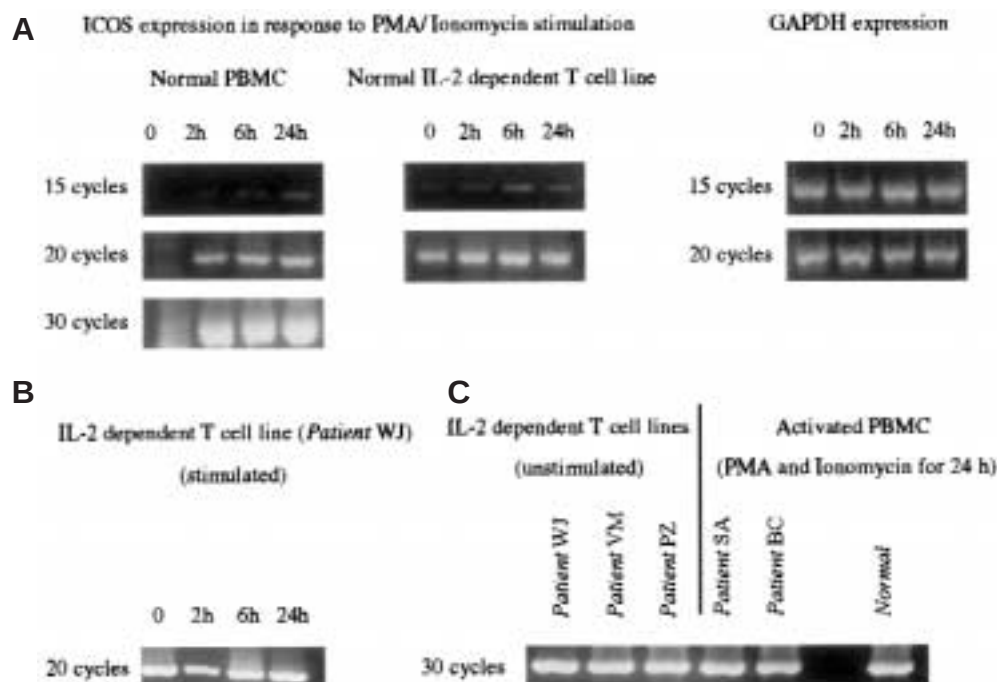


FIG 2. ICOS mRNA expression by activated PBMCs and IL-2-dependent T lymphoblasts. **A**, mRNA derived from resting or stimulated normal PBMCs and IL-2-dependent T lymphoblasts were semiquantitatively assessed by RT-PCR. ICOS mRNA was not detected in unstimulated PBMCs after 30 cycles (*left panel*) but was observed in IL-2-dependent T lymphoblasts even without additional stimulation (*middle panel*). GAPDH expression served as control (*right panel*). **B and C**, patients' IL-2-dependent T lymphoblasts and activated PBMCs from representative patients showed quantities of ICOS mRNA that were similar to those of normal controls.

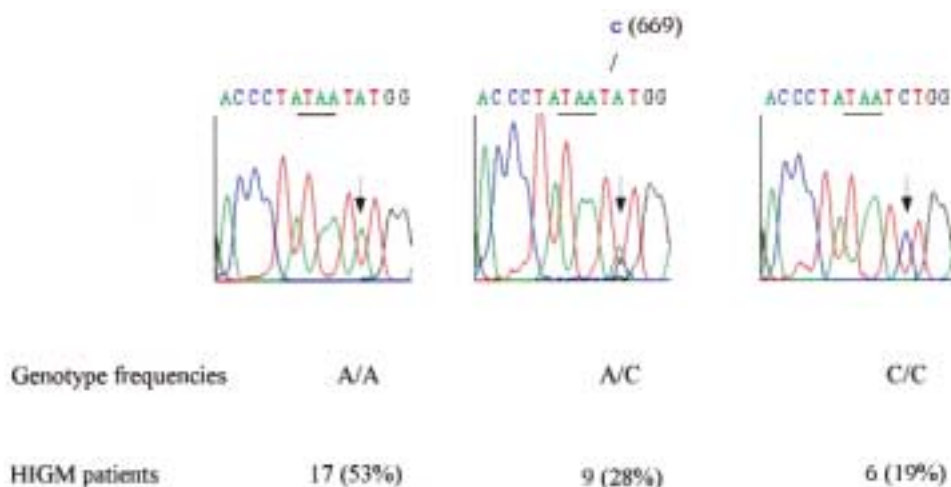


FIG 3. A polymorphism (T 669 C) downstream of the stop codon (TAA) was observed in controls and HIGM patients at a similar frequency.

mal dysplasia or incontinentia pigmenti and infections that are generally less severe.^{12,13,14}

As part of a long-term project to study the molecular basis of primary immunodeficiency diseases, we have collected blood from 136 patients from 113 unrelated families with the clinical and laboratory findings of HIGM. The molecular defect could be identified in 103

patients from 83 families. However, in 33 patients from 30 families, none of the 4 genes traditionally associated with HIGM were affected.

Because costimulatory molecules play an important role in T-lymphocyte activation and immune function, we explored the possibility that members of the CD28/CTLA-4 family, including the inducible costimula-

tory molecule ICOS, might be defective in HIGM patients. CD28, a receptor for B7 gene products, is required for the initiation of T-cell immune responses.²² CTLA-4, known to bind B7 with high affinity, is induced after T-cell activation and plays an important role in the downregulation of T-cell responses.²³ ICOS, the third member of the CD28/CTLA-4 family, is expressed by activated T cells; its ligand, B7RP-1 (ICOSL), is constitutively expressed by B cells, monocytes, and nonlymphoid tissues.¹⁵⁻¹⁹ ICOS is expressed by lymphocytes located in the T-cell zones of germinal centers, the site of terminal B-cell differentiation.¹⁶ When tested in vitro, both ICOS and CD28 were shown to effectively costimulate all basic T-cell responses, including proliferation, upregulation of molecules mediating cell-cell interaction, synthesis of cytokines, and effective T-cell help in antibody secretion.^{15,19} ICOS is particularly effective in releasing IL-10,^{15,24} a cytokine implicated in the initiation of B-cell memory and generation of plasma cells.^{25,26} The dominant expression of ICOS in germinal centers of lymphoid tissue suggests that this costimulatory molecule is essential for T-cell help in terminal B-cell differentiation.

The generation of ICOS-deficient mice allowed the systematic investigation of the function of ICOS. Whereas the lack of ICOS does not affect T- and B-cell development, ICOS^{-/-} T cells, when activated in vitro, have a profound deficiency of proliferation compared with wild-type T cells. The reduced proliferation of ICOS^{-/-} T cells is associated with a marked decrease in expression of the T-cell activation markers CD40L, CD25, and CD69.²⁷ A striking deficiency in humoral responses due to defective interaction between CD4⁺ T-helper cells and B cells was consistently observed. Reduced serum levels of IgG1, IgE, and, to a lesser degree, IgA suggested defective isotype switching.²⁷ In vitro and in vivo antibody responses to T-cell-dependent antigens were severely depressed in all systems tested.^{20,27} Furthermore, the formation of germinal centers, the anatomic hallmark for T-cell-dependent immune responses, and the physical site of T-/B-cell interaction was severely impaired in ICOS^{-/-} mice compared with wild-type mice.

Based on the observations made in ICOS^{-/-} mice, we decided to investigate ICOS expression in a subgroup of HIGM patients for whom a molecular diagnosis had not been established. Activated PBMCs or IL-2-dependent T-cell lines from all 33 patients studied expressed ICOS normally, and sequence analysis of the ICOS coding region revealed only wild-type sequences. A polymorphism (669 A/C) was observed 2 bp downstream of the termination codon. This polymorphism, described previously in normal subjects, was present in the HIGM cohort at the same allelic and genotypic frequencies as reported by Haimila et al.²⁸ We also did not notice any differences in the kinetics and quantity of ICOS transcription and expression. In vitro activation of normal control and patient primary T cells with PMA and ionomycin resulted in rapid expression of ICOS mRNA, which became detectable at 2 hours and was strongly expressed at 24 hours (Fig 2) when 84% to 98% of CD4⁺

cells expressed ICOS protein (Fig 1). A similar expression of ICOS was observed after activation with anti-CD3 monoclonal antibody or with PHA.^{16,29} The consistently strong expression of ICOS in the IL-2-dependent T-cell lines derived from patients and normal subjects suggests that ICOS might play a role in the maintenance and proliferation of T lymphoblasts.

There are several possibilities to explain the negative finding. The lack of ICOS in mice might result in a different phenotype than in humans. Mice might be more dependent on the interaction of ICOS with its ligand for maturation of B cells, the development of B memory cells, and CSR. In human, ICOS might be a less important costimulator than CD40L, the latter being absolutely required for CSR and somatic hypermutation. It is also possible that our sample number was too small or that we studied the wrong syndrome. Grimbacher and coworkers³⁰ have recently identified 4 patients with the clinical diagnosis of common variable immunodeficiency (CVID) from 2 families with a large deletion of ICOS, resulting in a frame shift and truncation of the molecule. Although reportedly not related, the affected members of the 2 families are homozygous for the same deletion and have lived for many generations in the same region of the Black Forest. The ICOS-deficient patients, all with late-onset symptoms, had low IgG, IgA, and IgM, except for 1 patient who had normal IgM as a teenager. B-cell numbers were decreased, and memory B cells were absent. Interestingly, when patient B cells were cocultured with autologous T cells in the presence of IL-2 and *Staphylococcus aureus* Cowan I, all IgG isotypes were detected. This suggests that the lack of ICOS can be, at least in part, compensated for by other costimulatory molecules. Grimbacher and colleagues hypothesized that switch recombination is not strictly dependent on ICOS/ICOSL interaction but rather augmented by ICOS-mediated signals. Thus, in humans, ICOS differs from CD40L expressed by activated T cells, whose interaction with CD40 on B cells is an absolute requirement for CSR.⁴ This incomplete block of B-cell signaling would explain why ICOS-deficient patients develop symptoms later in life and that their immune defect is rather limited to the failure of generating or expanding memory B cells and long-lived plasma cells.³⁰

The majority of the patients reported here resemble CVID patients; some presented early in life, others had late onset. All had a history of recurrent respiratory infections, and many developed bronchiectasis. They had in common hypogammaglobulinemia except for IgM, and, as is characteristic of CVID patients, their lymphocytes often proliferated poorly in response to antigenic stimulation.³¹ Failure to isotype switch is not unusual for CVID patients, suggesting a defect of either T-cell activation or of B-cell maturation. Accordingly, patients with the clinical and laboratory findings of HIGM or CVID without a known molecular diagnosis are excellent models in whom to search for the identification of new genes that are critical for T-/B-cell interaction, B-cell signaling, CSR, and somatic hypermutation.

We appreciate the willingness of the patients and their families to participate in these research studies. We thank the physicians for their referral assistance and Marge Young for technical support.

Since this manuscript was accepted for publication, one of the 33 patients reported, a 39-year-old man of Middle Eastern descent, was found by Wen-I Lee, MD, to have a homozygous mutation of Uracil-DNA glycosylase (*UNG*), a gene required for CSR and somatic hypermutation.³²

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