Somatic Mutations in Human Ig Variable Genes Correlate with a Partially Functional CD40-Ligand in the X-Linked Hyper-IgM Syndrome

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X-linked hyper-IgM (HIGM-1) syndrome is a rare disorder resulting from mutations in the CD40-ligand (CD40L) gene. This defect is associated with normal or elevated serum levels of IgM, and with low to undetectable levels of serum IgG, IgA, and IgE. We analyzed the somatic mutation status in Ig V genes from three unrelated HIGM-1 patients by reverse-transcription PCR and sequence analysis. Two patients (B.S. and P.S.) expressed unmutated V_H6 genes. In contrast, one patient (A.T.) was found to express mutated V_H6 genes. Whether the presence of somatic mutations in this patient was related to a functional CD40L was assessed by deriving T cell clones from his peripheral blood cells. Upon activation, these T cell clones expressed weakly and transiently surface CD40L, and were able to induce limited isotype switch of normal naive B cells, indicating residual CD40L function. Altogether, our results 1) confirm the central role played by CD40L in the generation of somatic mutation (patients B.S. and P.S.), 2) provide an unusual illustration of the relative dissociation between somatic mutation and isotype switching (patient A.T.), and 3) demonstrate a further complexity of the X-linked HIGM syndrome that may occur despite a partially functional CD40L. *The Journal of Immunology,* **1996, 157: 1492–1498.**

One of the central features of the immune system is the production of memory T and B cells. Memory B cells are characterized by their rapid secretion of high affinity Abs, mainly of the non-IgM isotypes. The events leading to the generation of memory B cells occur in the germinal center and include somatic mutation, clonal expansion, Ag-driven selection, and isotype switching (1–9). T cell/B cell interactions comprise both cytokines and contact-mediated signals, of which CD40/CD40L[3] interaction is critically important (9–14). CD40L, a type II transmembrane glycoprotein belonging to the TNF superfamily, is mostly expressed on activated $CD4^+$ T cells (15–20). Both in vivo and in vitro studies have documented that T cell-dependent isotype switching is dependent strictly on CD40/CD40L interaction (21–24). Moreover, in vivo studies have demonstrated that the impairment of the CD40/CD40L interaction inhibits germinal center full development and generation of memory B cells (22–25). X-linked HIGM-1 syndrome is a rare immunodeficiency disorder resulting from a structural alteration (point mutation, deletion, or truncation) of the CD40L coding gene (10, 26–32). Affected patients fail to generate normal memory B cell responses and have low to undetectable levels of serum IgG, IgA, and IgE, but normal or elevated levels of IgM. These individuals suffer from recurrent opportunistic and pyogenic infections. Moreover, histologic studies have reported the absence of germinal centers in the secondary lymphoid organs of these patients (33, 34). The lack of germinal centers in such patients led us to hypothesize that they should only produce unmutated Igs. Herein, we studied the in vivo occurrence of somatic mutations in V_H5 and V_H6 genes from B cells isolated from three unrelated X-linked HIGM-1 patients. Ig transcripts of two patients (B.S. and P.S.) were indeed in germ-line configuration, but surprisingly, one patient (A.T.) had mutated Ig transcripts. To determine whether patient A.T. displayed a functional CD40L, T cell clones were generated from his peripheral blood, and the function of their CD40L was examined in vitro. Weak and transient expression of surface CD40L was detected in these T cell clones, which could indeed induce naive B cells to undergo isotype switching in a CD40L-dependent fashion, but to a lesser extent than a normal control.

Therefore, our data demonstrate that a diminished and shortened expression of CD40L results in an HIGM syndrome, even though a proportion of B cells can be induced to undergo somatic mutation.

Materials and Methods

Selection of the X-linked HIGM-1 patients

Three unrelated X-linked HIGM-1 patients were selected for somatic mutation analysis. Patients B.S. and P.S. have been described previously (35). Patient A.T. was classified previously as type I, according to the absence of staining of his activated T cells with the CD40-Ig, 5C8, and anti-TRAP antiserum (26, 35).

RNA isolation and reverse transcription

Total RNA was extracted from 2 to 10×10^5 PBL by a single step method using guanidium thiocyanate/phenol/chloroform (36). Total RNA was reverse transcribed into cDNA using a random hexamer primer (Pharmacia, Uppsala, Sweden) and SuperScript $RNaseH^-$ reverse transcriptase (Life

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[3] Abbreviations used in this paper: CD40L, CD40 ligand; aa, amino acid; CDR, complementarity-determining region; FR, framework; HIGM, hyperimmunoglobulin M; PE, phycoerythrin; R/S, ratio of replacement to translationally silent mutation; sIg, surface immunoglobulin; TRAP, TNF-related activation protein; ND, normal donor.

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Table I. *Clinical features of the HIGM patients*

	Age of Diagnosis	Age	Infections	Family History	Related Problems
BS	2 years	18 years	Pulmonary Bronchiectasis	No	Heart failure
PS	3 months	3 years	Pertussis Pneumonia	Yes 2 affected male siblings 1 died at 5 months of age	None known
AT	18 months	31 years	Pulmonary Chicken pox Measles Otitis media Gastroenteritis Pyelonephritis Fungal infections	Yes 3 male deaths in infancy mothers family Male cousin died at 20 years of age	Biliary disease Polyarthritis Lymphadenopathy Follicular hyperplasia

Technologies, Bethesda, MD) in a 20-μl final volume. First strand cDNA (3 μl) was used directly for PCR amplification.

PCR amplification

PCR reactions were conducted for 35 cycles under standard conditions (preheating 3 min at 80°C, denaturation 1 min at 94°C, annealing 2 min at 65°C, extension 3 min at 72°C) in a final volume of 100 μl containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin, 200 μM of each dNTP, 2.5 U *Taq* polymerase (*GeneAmp* PCR reagents kit; Perkin-Elmer Cetus, Norwalk, CT), 5% DMSO, and 100 ng of each amplification primer (Appligene, Illkirch, France). Oligonucleotide primers used for the PCR amplification were:

5′-CD40L, GGATCCAGAAGATACCATTTCAACTT;
3′-CD40L, GAAGATCTTCGGATCCTGCAAGGTGACACTGTTCAG;
Cμ, TGGGGCGGATGCACTCCC;
5′-V_H6, CCCGAATTCATGTCTGTCTCCTTCCTCAT;
3′-V_H6, GCACAGTAATACACAGCC;
5′-V_H5, CCCGAATTCATGGGGTCAACCGCCATCCT.

Purification and subcloning of the PCR products

The PCR products were size fractionated by electrophoresis in 1% low melting point NuSieve GTG agarose gels (FMC Bioproducts, Rockland, MD) and recovered after gelase treatment (Epicentre Technologies Corp., Madison, WI). The purified PCR products were ligated into an *Eco*RV-digested and ddT-tailed Bluescript vector (pBluescript; Stratagene, La Jolla, CA). The ligation mixtures were transformed in MAX Efficiency DH5α competent cells (Life Technologies). Bacteria were grown in Luria-Bertani medium supplemented with 50 μg/ml X-galactoside, 1 mM isopropylthio-β-D-galactoside; and 100 μg/ml ampicillin. Recombinant plasmids were extracted using a plasmid mini kit (Qiagen, Chatsworth, CA), tested by restriction analysis, and sequenced on both strands using *Taq* DyeDeoxy Terminator cycle sequencing kit and a 373 DNA automatic sequencer (both from Applied Biosystems, Foster City, CA).

Analysis of DNA sequences

The mutation in the CD40L coding gene was determined for patients B.S. and P.S., and confirmed for the previously described patient A.T. (26). Ig sequences were compared with the GenBank data library and analyzed with the Geneman program (DNAStar, Madison, WI).

Generation of T cell clones from patient A.T.

$CD4^+$ T cell clones were generated from peripheral blood of patient A.T. by a random cloning method (37). The generation of the $CD4^+$ T cell clone MT9, expressing a wild-type CD40L, has been reported previously (20).

Reagents

CD40 Ab mAb89 (IgG1) was described before (12). mAb LL2 (IgG1) directed against CD40L was generated by immunization with CD40L-transfected L cells, described elsewhere (38), of syngeneic mice (C3H/HeJ) and fusion with SP2/0. PHA was purchased from Wellcome (High Wycombe, U.K.), and rIL-2 from Amgen (Thousand Oaks, CA), while rIL-10 was provided by Schering-Plough, RI (Kenilworth, NJ).

Culture conditions

T cell clones were expanded in Iscove's derived medium (39), supplemented with 5% FCS, and stimulated weekly with a feeder cell mixture containing irradiated PBL, irradiated lymphoblastoid cell line JY, 0.1 μg/ml of PHA, and 20 U/ml of IL-2. T cell clones were used 1 wk after the last stimulation with feeder cells, as described (37). L cells were cultured in RPMI 1640, and supplemented with 10% FCS, glutamine, and antibiotics.

B/T cell coculture conditions

$sIgD^+$ B cells were purified from human tonsils, as described before (39). IgD was expressed on >99% of the $sIgD^+$ B cell subpopulation, as assessed by fluorescence analysis using a FACScan (Becton Dickinson, Palo Alto, CA). Microculture wells (Falcon, Lincoln Park, NJ) were coated with anti-CD3 Ab OKT3 (1/1000 diluted ascites) for at least 2 h at room temperature. 2×10^5 irradiated (30 Gy) cloned T cells and 2×10^4 B cells were added per well. As control, $sIgD^+$ B cells were cultured with the same combination of cytokines and T cell clones in the absence of CD3 stimulation. After 14 days of culture, supernatants were harvested and tested in isotype-specific ELISAs.

B cell proliferation assay

B cells were cultured in triplicate at a concentration of 2×10^4 cells per 200 μl in 96-well flat-bottom plates (Falcon). For T/B cocultures, wells were coated with anti-CD3 Ab OKT3 (1/1000 diluted ascites) for at least 2 h, and irradiated (30 Gy) cloned T cells were added at a concentration of 2×10^5/well. Proliferation was determined after different times of culture, as indicated in the legends, by addition of [^{3}H]thymidine (1 μCi/well, 25 Ci/mM; DuPont NEN, Boston, MA) during the last 16 h of culture. [^{3}H]Thymidine incorporation was determined by liquid scintillation counter.

FACS analysis

Two different methods were used for surface CD40L staining: 1) cells were stained with an anti-TRAP antiserum (26), followed by biotinylated goat anti-rabbit Ab (1/100 dilution; Dakopatts, Glostrup, Denmark) and streptavidin PE (Becton Dickinson, Mountain View, CA); and 2) cells were stained with biotinylated mAb LL2 (1 μg/ml), followed by streptavidin PE. For cytoplasmic CD40L detection, cells were permeabilized with 0.33% saponin (Sigma Chemical Co., St. Louis, MO) before staining, and all subsequent steps were performed in the presence of saponin. Cells were analyzed with a FACScan flow cytometer (Becton Dickinson).

Results

Clinical status of three X-linked HIGM-1 patients

The clinical features of the patients included in this study are summarized in Table I. They all suffered from recurrent episodes of pyogenic infections during early infancy, after which the diagnosis of X-linked HIGM disease was made. They all received regular i.v. injections of Igs, and antibiotics when needed. While patients A.T. and P.S. had close male relatives who died young from infections, patient B.S. developed a sporadic form of the disease. Clinical examination of patient A.T. revealed generalized lymphadenopathy, a finding commonly associated with this disorder (35). The diagnosis of X-linked HIGM was confirmed by sequencing the CD40L coding gene from each patient (Fig. 1*A*), including patient A.T., who was previously reported (26). Mutations affected the

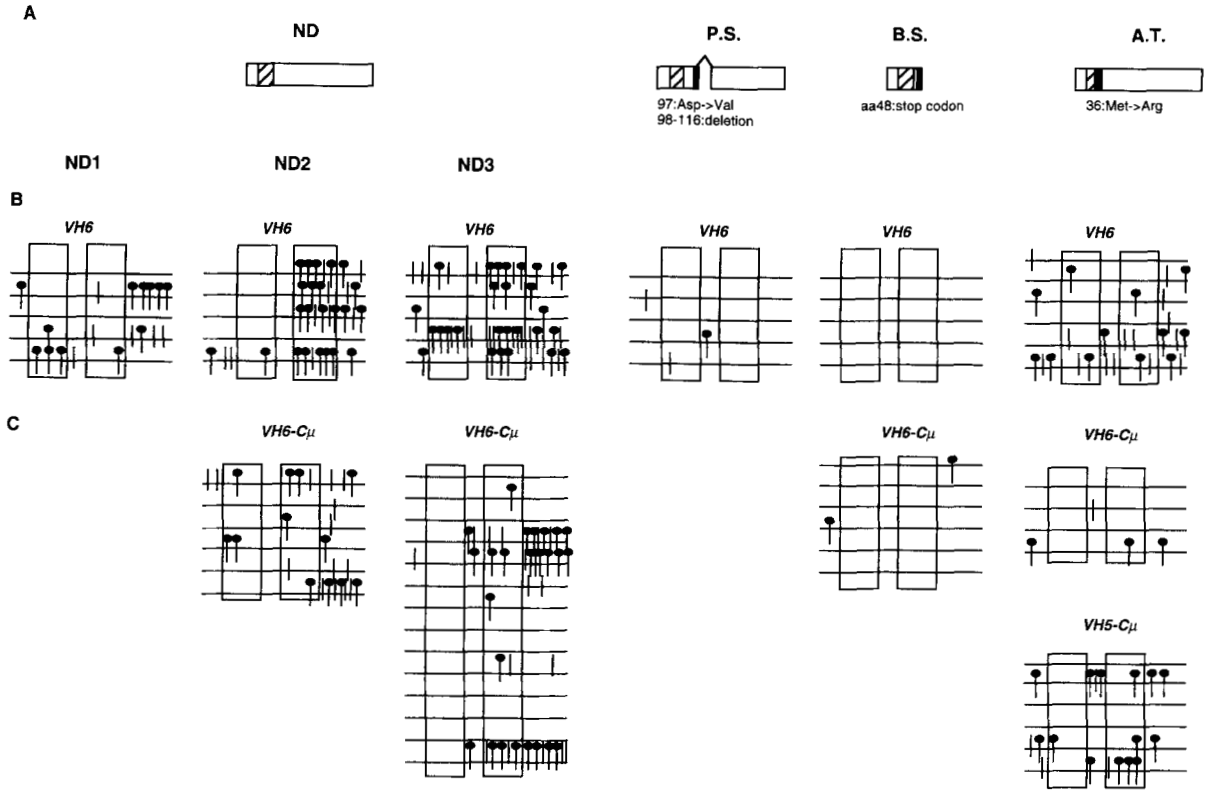

FIGURE 1. CD40L and V_H sequences. *A*, Schematic representations of the CD40L cDNA sequences from normal donor (ND) and HIGM-1 patients (P.S., B.S., A.T.). The transmembrane domain is represented by hatched box. The location of the mutation in the CD40L coding gene is represented in the black box, and specified below. *B* and *C*, Schematic representations of the V_H6 from normal donors (ND) and HIGM-1 patients (*B*), and V_H6-Cμ sequences from normal donors (ND2 and ND3), and V_H6-Cμ and V_H5-Cμ for HIGM-1 patients (*C*). Replacement and silent mutations are localized within the sequences and figured as (†) and (|), respectively. The CDR are boxed.

extracellular CD40 binding domain of patients B.S. and P.S., causing nucleotide deletions. In B.S., this resulted in a frame shift that introduced a stop codon at aa 48. In P.S., this yielded a replacement (97 Asp to Val), followed by deletion from aa 98 to 116. As previously described, the mutation of patient A.T. results in a single amino acid substitution (36 Met to Arg) that introduces a charged residue in the CD40L transmembrane domain (26).

The V_H6 and V_H5 transcripts from two X-linked HIGM-1 patients are germ line

To facilitate the detection of somatic mutations, the nonpolymorphic V_H6 gene was first analyzed. Transcripts were amplified with a leader V_H6-specific 5′ primer and with either a framework (FR) 3 V_H6-specific 3′ primer, or a μ-specific 3′ primer. After subcloning of the amplification products, several plasmids were sequenced for each patient.

Ten V_H6 clones (274 bp/clone) obtained from patients B.S. and P.S. were analyzed (Fig. 1*B*). Seven sequences were germ line, while three showed a single mutation. Among 21 independent V_H6/V_H5-Cμ rearrangements, 14 sequences were germ line and 7 displayed 1 or 2 mutations per sequence (Fig. 1*C*). This low frequency of mutations (3 mutations/2740 bp) is indistinguishable from the *Taq* error background (1 mutation/1000 bp).

The V_H5 and V_H6 transcripts from patient A.T. display somatic mutations

Six of six V_H6 sequences derived from patient A.T. were mutated, bearing from 1 to 11 mutations (Fig. 1*B*). This mutation frequency (26 mutations/1644 bp analyzed, 1.6%) fell within the normal range

Table II. *V_H6 somatic mutations of patient A.T.*

A. Frequency of somatic mutations[a]

	FRs	CDRs	V_H6
AT	1.38	1.9	1.6
ND1	1.07	1.9	1.3
ND2	1.07	5.9	2.4
ND3	2.15	6.66	3.4

B. R/S ratio[b]

	FRs	CDRs	V_H6
AT	0.7	1.2	0.85
ND1	1.2	2.5	1.57
ND2	1.2	3.4	2.30
ND3	0.8	2.1	1.35

[a] Frequency of mutations was calculated by summing all of the nucleotide changes from the germ line V_H6 and dividing by the total number of bases sequenced for specified portions of the V_H6 gene segment.

[b] R/S ratio represents the number of replacement changes divided by the number of silent changes for specified portions of the V_H6 gene segment.

(1.3–3.4%) established for 15 V_H6 clones isolated from three control individuals (ND1, ND2, and ND3; Fig. 1*B*, *left panel*). Mutations in sequences from patient A.T. were distributed evenly between the complementarity-determining regions (CDRs) (9/450 bp, 2%) and the FR (17/944 bp, 1.8%) regions, while mutations in sequences from controls have preferentially accumulated in CDRs (54/1125 bp, 4.8%) vs FRs (43/2730 bp, 1.6%) (Table II*A*). The replacement vs silent ratio of mutations (R/S) was low (4/5, R/S = 0.8) in the CDRs from

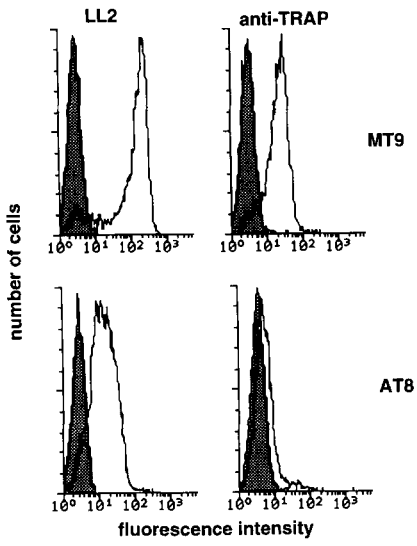

FIGURE 2. Expression of CD40L on HIGM-1 T cell clone A.T.8. $CD4^+CD40L$ control T cell clone (MT9) and HIGM-1-derived T cell clone (A.T.8) (10^6 cells/ml) were stimulated with the combination of PMA (10 ng/ml) and ionomycin (1 μg/ml) for 3 h. Surface labeling of the T cells was obtained by staining with biotinylated anti-CD40L mAb, LL2 at 1 μg/ml, or a polyclonal anti-TRAP antiserum, followed by biotinylated goat anti-rabbit Ab, and revealed by streptavidin PE (open profile). The control was performed by staining with an irrelevant IgG1-biotinylated control mAb, followed by streptavidin PE (solid profile).

patient A.T. when compared with the three controls (39/15, R/S = 2.6) and with the expected R/S ratio of human V_H6 CDRs (R/S = 3.9) (40).

We next analyzed 4 V_H6-μ and 6 V_H5-μ transcripts from patient A.T. and 20 V_H6-μ transcripts from two normal individuals for the presence of somatic mutations (Fig. 1*C*). Among 10 V_H5/V_H6-μ sequences derived from A.T., 5 were germ line, while the other 5 had accumulated from 1 to 7 mutations. Similarly, 7 V_H6-μ transcripts derived from controls were germ line, while the 13 others had accumulated from 1 to 13 mutations. The overall frequency of mutation in the μ transcripts was 0.8% (22/2740 bp) for patient A.T. compared with 1.2% (68/5480 bp) for the controls.

Activated T cells from patient A.T. transiently express surface CD40L

The presence of somatic mutations in the V_H gene segments from patient A.T. raised the question as to whether his CD40L molecule, despite the point mutation in its transmembrane domain, was still functional. Five T cell clones derived from the PBL of patient A.T. were found negative for CD40L when stained with the anti-TRAP antiserum, even after activation with PMA plus ionomycin, as previously described for activated PBL from this patient (26). In contrast, LL2, an anti-CD40L mAb recently generated in our laboratory, detected surface expression of CD40L by A.T.8 T cell clone after treatment with PMA and ionomycin (Fig. 2). Kinetic analysis on A.T.8 cells revealed that CD40L surface expression was detectable 3 h after stimulation and disappeared after 16 h (Fig. 3). A similar transient expression of CD40L by A.T.8 was also observed upon anti-CD3 activation (Fig. 3). Interestingly, cytoplasmic expression of CD40L was already detected 1 h after activation, and lasted for a longer period than the surface expression (Fig. 3). As expected, staining with mAb LL2 showed a CD40L surface expression by a control $CD4^+$ T cell clone, MT9, but with different kinetics: after activation with PMA and ionomycin (Fig. 3), CD40L surface expression peaked between 3 and 10 h and was still detectable after 24 h. Of note, the signal of staining with mAb LL2 was significantly stronger than with the anti-TRAP antiserum (Fig. 2, *top panel*). Thus, a transient expression of CD40L was detectable on the T cell clone from patient A.T., provided that 1) early time points after activation were analyzed, and 2) an anti-CD40L mAb was used instead of the anti-TRAP antiserum.

T cell clones from patient A.T. induce low levels of CD40L-dependent proliferation and IgG production by normal naive $sIgD^+$ B cells

We tested whether the transiently expressed CD40L from patient A.T. was functional in vitro. Naive tonsillar $sIgD^+$ B cells were cocultured with anti-CD3-activated A.T.8 T cells, or with MT9 control T cells. Activated MT9 cells induced strong DNA synthesis in $sIgD^+$ cells, which was further enhanced by addition of IL-2 and IL-10 (Fig. 4). Both anti-CD40L and anti-CD40 blocking mAbs strongly inhibited this proliferation. Of note, the naive B cell proliferation induced by A.T.8 clone was always weaker (ranging from 25 to 50% of the response with MT9 T cells), and was reduced only slightly by addition of anti-CD40 or anti-CD40L mAbs, indicating that it resulted mainly from a CD40/CD40L-independent T/B interaction.

As expected, $sIgD^+$ B cells produced high levels of IgM (120 μg/ml) and of IgG (27 μg/ml) when cultured with the activated MT9 cells, in the presence of exogeneous IL-2 and IL-10 (Fig. 5, *upper panel*). Addition of the blocking anti-CD40L mAb (LL2) strongly and specifically inhibited IgM and IgG production, while a control mAb had no effect. $sIgD^+$ B cells cultured with anti-CD3-activated A.T.8 cells, in the presence of exogeneous cytokines, secreted low but significant levels of IgM (2.9 μg/ml) and IgG (1.2 μg/ml) (Fig. 5, *lower panel*). Addition of the anti-CD40L mAb LL2 almost entirely blocked the IgG production, therefore demonstrating that the CD40L from patient A.T. was at least partially functional.

Discussion

We analyzed the somatic mutation in the Ig V gene segments of three patients with X-linked HIGM-1 syndrome. Two patients possessed CD40L molecules that have almost entirely (patient B.S.) or partially (patient P.S.) lost their extracellular domain, and they expressed unmutated rearranged V_H6 transcripts. Inasmuch as somatic mutation occurs during the proliferation of centroblasts within the dark zone of germinal centers (3, 6, 41), this observation is in agreement with the reported lack of germinal centers in X-linked HIGM-1 patients (33, 34), as well as in CD40- or CD40L-deficient mice (22–24).

In contrast, somatic mutations were present in the V_H6 and V_H5 transcripts of patient A.T., with a frequency comparable with that observed in normal controls. Somatic mutations were distributed evenly along the V_H sequences from patient A.T. However, no conclusion can be drawn regarding the Ag-driven selection process because the R/S ratios were similarly low in the CDRs and FRs of V_H sequences derived from A.T. and from controls (2, 4, 42–44). The lower frequency of mutations in the V_H6-μ vs V_H6 transcripts from both patient A.T. and control individuals (0.8 vs 1.6% and 1.2 vs 2.4%, respectively) suggested that at least part of the V_H6 transcripts was associated with secondary isotypes in both cases, but it was not possible to directly confirm this hypothesis; while γ and α transcripts were amplified after 35 cycles of PCR from the PBL of

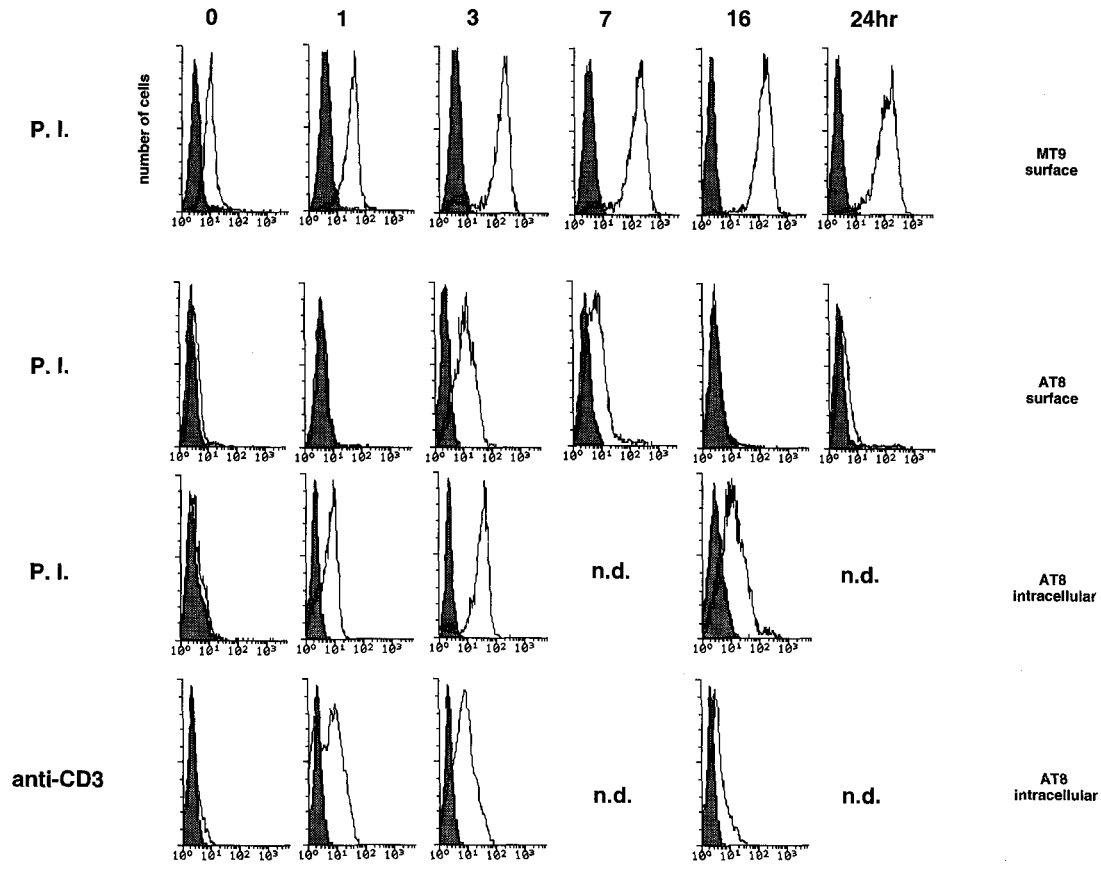

FIGURE 3. Kinetics of surface and intracellular expression of CD40L on A.T.8. $CD4^+CD40L$ control T cell clone (MT9) and HIGM-1-derived T cell clone (A.T.8) (10^6 cells/ml) were stimulated with the combination of PMA (10 ng/ml) and ionomycin (1 μg/ml) (P.I.) or anti-CD3 (1/1000 diluted ascites) at various time points, as indicated on *top* of the figure. Surface staining was performed, as indicated above, with anti-CD40L LL2 mAb (*upper two panels*). For cytoplasmic detection of CD40L (*lower panel*), T cells were permeabilized with 0.33% saponin and stained with LL2 mAb, as stated above.

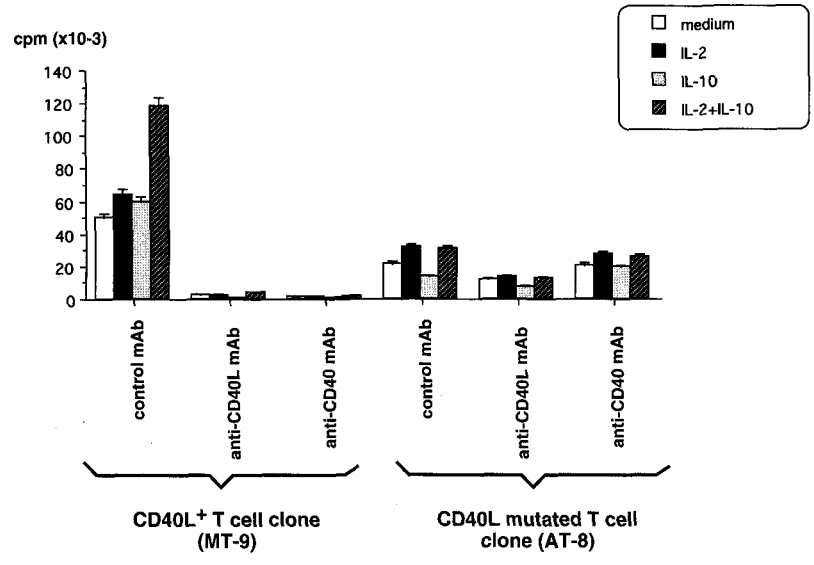

FIGURE 4. HIGM-1 T cell clone induces low levels of B cell proliferation. 2×10^4 $sIgD^+$ naive B cells were cultured with 2×10^5 irradiated control T cell clone (MT9) or HIGM-1 T cell clone (A.T.8) in anti-CD3-precoated wells. Anti-CD40 Ab, mAb89 or anti-CD40L mAb, and LL2 or an isotype-matched control mAb were used at 1 μg/ml and added at the onset of the culture with or without 20 U/ml of IL-2, 100 ng/ml of IL-10, or combination of IL-2 and IL-10. [^{3}H]Thymidine incorporation was measured at day 6. Data are expressed as mean of triplicate culture. Representative of three separate experiments.

patient A.T. using a V_H consensus 5′ primer (data not shown), neither V_H5-Cγ nor V_H5-Cα PCR products could be obtained.

The presence of somatic mutation exclusively in patient A.T. raised the question about a residual function of his CD40L. Indeed, A.T. is the only X-linked HIGM-1 patient reported to date to have a mutation (36 Met to Arg) in the transmembrane domain of his CD40L. This mutation was discussed previously as destabilizing the surface expression of the CD40L molecule. In keeping with this, charged residues in the transmembrane domain of molecules such as TCR or sIg individual chains need to be masked to insure their proper surface expression (45, 46). As originally described for peripheral blood T cells (26), the anti-TRAP antiserum did not

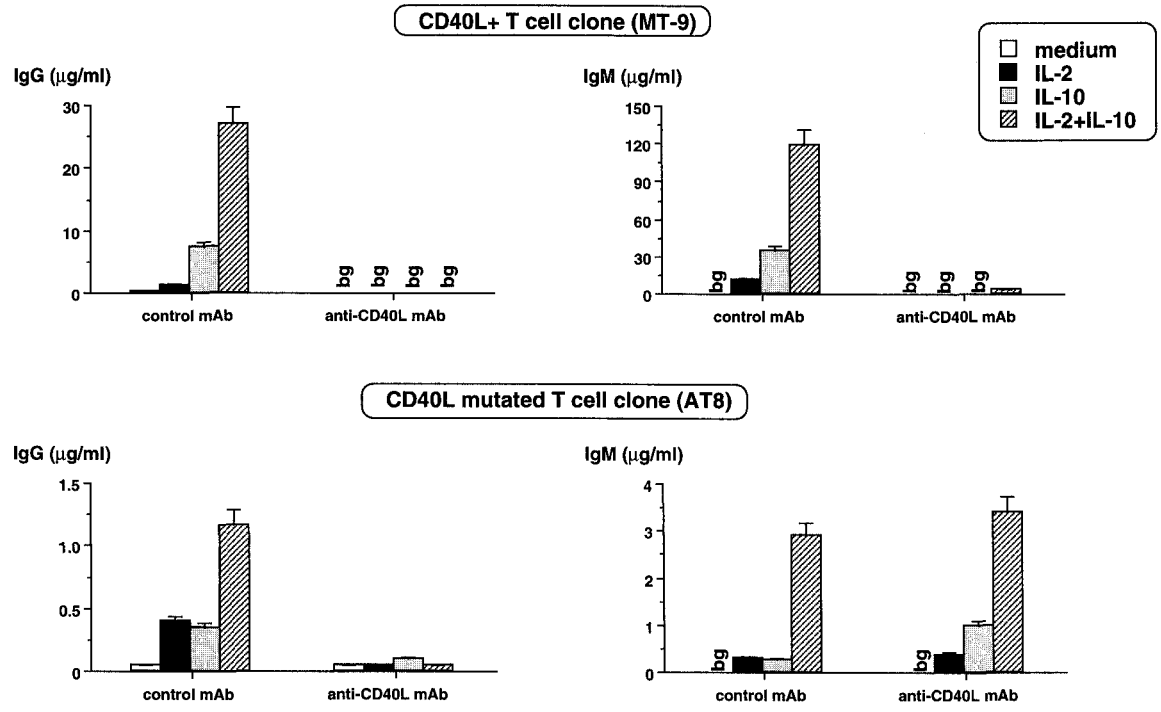

FIGURE 5. HIGM-1 T cell clone-induced IgG production by naive B cells. sIgD$^+$ naive B cells were cocultured with irradiated control T cell clone (MT9) or HIGM-1 T cell clone (A.T.8), as described above. Anti-CD40 Ab, mAb89 or anti-CD40L mAb, and LL2 or an isotype-matched control mAb were used at 1 μg/ml and added at the onset of the culture with or without 20 U/ml of IL-2, 100 ng/ml of IL-10, or combination of IL-2 and IL-10. Supernatants were harvested after 12 days of culture, and their IgG and IgM contents were measured in ELISA. Data are expressed as mean of triplicate culture. Representative of three separate experiments. Levels of Ig measured in supernatants of sIgD$^+$ B cells cocultured with T cell clones in the absence of CD3 stimulation were below detection sensitivity of ELISAs (10 and 40 ng/ml for IgG and IgM, respectively) (data not shown).

allow the detection of CD40L on the surface of several CD4$^+$ cells derived from this patient. However, LL2, a mAb directed against human CD40L, clearly detected a very transient expression of surface CD40L by cloned T cells (A.T.8) after activation. The discrepancy with the previous report can be explained by 1) the differences in the reagents used to stain CD40L, using a mAb (LL2, generated against the full molecule) or a polyclonal antiserum (anti-TRAP generated against a peptide aa 38 to 183 (26)); 2) the higher level of CD40L expression on activated T cell clones compared with freshly isolated T cells (20, 47); and 3) the down-regulation of CD40L expression on PBL in the presence of B cells (48). Our data demonstrate that the mutation in the transmembrane domain of CD40L from patient A.T. does not result in a complete suppression, but rather in a significant reduction of both the level and the duration of CD40L expression on the T cell surface after activation.

To assess the functionality of this CD40L, we tested the ability of A.T.8 T cells to induce isotype switching of sIgD$^+$ naive B cells (39). We found that activated A.T.8 T cells induced a moderate but significant IgG secretion, as well as a weak proliferation by sIgD$^+$ naive B cells. Addition of anti-CD40L mAb almost completely inhibited the IgG secretion, demonstrating a CD40L activity. In contrast, the proliferation was affected only slightly by the blocking anti-CD40L mAb, which suggests the existence of a compensatory CD40/CD40L-independent proliferation pathway in such HIGM patient.

Our findings indicate that the presence of somatic mutation correlates with the expression of a partially functional CD40L. Recently, low levels of somatic mutations in V_H genes have been reported in two of three X-linked HIGM-1 patients, but the possible CD40L activity was not assessed (49).

Interestingly, recent studies have highlighted the roles of CD40 vs CD40L signaling in B and T cell activation, respectively (25, 50, 51). CD40 triggering of the B cells plays a major role in the induction of isotype switching (51, 52), and is involved in the generation of memory B cell development (25, 51). On the other hand, it has been shown that CD40L engagement enables T cells to initiate (51) and to continue germinal center reaction (25). Therefore, how the absence (or reduction) of CD40L signaling on T cells participates in the immune defect of X-linked HIGM-1 patients remains an open and intriguing question.

An important conclusion from our study is the demonstration of a molecular heterogeneity among X-linked HIGM-1 patients that is reflected by the presence or absence of somatic mutation in their Ig V_H genes. The limited number of patients in this study did not permit us to establish a correlation between the extent of the CD40L defect and the severity of the disease. Nevertheless, our data suggest that the outcome of the CD40/CD40L interaction on B cell might depend on its duration and its intensity. While a transient interaction could be sufficient to trigger somatic mutation, a more prolonged and intense engagement of the two molecules might be required for isotype switching to occur efficiently.

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