

CD154 DEFICIENCY AND RELATED SYNDROMES

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PHYSIOLOGY OF THE CD40-CD154 SYSTEM

The relationship between the cell surface molecule CD40 and its ligand CD154 underlies numerous associations between cells of the innate and adaptive immune systems. A glance at the variety of cell types expressing these molecule (Table 1) already hints at the diversity of interactions that may be influenced by the mutual ligation of CD40 and CD154.

CD40 was described initially in the mid-1980s as a B-cell surface glycoprotein with a role in regulating the cell cycle.²⁵ CD40 was soon shown also to regulate immunoglobulin (Ig) production and class-switching.¹⁵¹ Its ligand, which has been named gp39, T cell-B cell activating molecule (T-BAM), tumor necrosis factor (TNF)-related activation protein (TRAP), CD40 ligand (CD40L), and now carries the cluster designation CD154, was initially identified on activated T cells.⁵³ In 1993, several groups reported that the defect in the primary immune deficiency disease X-linked hyper-IgM (X-HIM) syndrome was due to mutations in the *CD154* gene.^{4, 9, 32, 42, 82} This finding clearly established the importance of CD154 and CD40 for the process of Ig class-switching (see later discussion) and led to an explosion in research of this intercellular signaling system.

CD154 and CD40 belong to large and growing cytokine and cytokine receptor families. CD154 is a member of the TNF family of cytokines; CD40 is within the family of TNF receptors (TNFR).⁵⁵ CD154 is a type II transmembrane protein with its C terminus outside the cell; its

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Table 1. CELLULAR DISTRIBUTION OF CD154 AND CD40

Cells Expressing CD154	Cells Expressing CD40
Activated T cells ⁵³	CD34 ⁺ hemopoietic precursors ³⁹
B cells ⁵⁴	B cells ²⁵
Mast cells ⁴⁷	T cells ¹⁴⁴
Basophils ⁴⁷	Monocytes ³
Eosinophils ⁴⁶	Macrophages ³
Natural killer cells ¹⁷	Dendritic cells ¹⁹
Monocytes ⁹¹	Langerhans' cells ¹⁹
Macrophages ⁹¹	Eosinophils ¹⁰²
Dendritic cells ¹¹²	Endothelial cells ⁶⁷
Endothelial cells ⁹¹	Thymic epithelium ⁴⁴
Vascular smooth muscle ⁹¹	Kidney epithelium ¹⁴²
	Keratinocytes ³⁰
	Carcinomas ¹⁴⁸
	Fibroblasts ¹²
	Hepatocytes ¹
	Vascular smooth muscle ¹²⁶

crystal structure has been determined.⁷⁶ The molecule is expressed on the cell surface in a trimeric form, analogous to TNF, and can bind simultaneously to three separate molecules of CD40. CD154 is expressed predominantly on activated CD4⁺ T cells,¹²² although it has been identified on some CD8⁺ T cells⁸⁴ and on some $\gamma\delta$ T cells.¹²² T-cell activation leads to transient expression of CD154.^{84, 122} Expression peaks within 6 to 10 hours depending on the mode of stimulation and is rapidly lost after 24 hours. Additional cells expressing CD154 are listed in Table 1. CD40 is a type I transmembrane protein; it is the only known ligand for CD154. Its cellular distribution is also listed in Table 1.

Numerous studies have investigated the consequences of CD40 ligation on B cells by various means. Binding CD40 with monoclonal antibodies induces leukocyte function antigen-1 (LFA-1)-dependent homotypic adhesion and, together with anti-IgM, causes proliferation.²⁴ CD40 ligation in the presence of interleukin (IL)-4 leads to IgE production.¹⁵¹ Cells expressing CD154 in the presence of IL-2 or IL-10 promote IgM, IgG, and IgA secretion.⁸ Resting B cells that receive signals through CD40 up-regulate IL-2, IL-4, and IL-5 receptors⁵² and the T-cell costimulatory molecules CD80 (B7-1) and CD86 (B7-2).⁶⁴ CD154 appears to have a dual role in regulating both the expansion and deletion of B cells.¹¹⁵ Activated T cells bearing both CD154 and the Fas (CD95) ligand (FasL) promote B-cell expansion if there is simultaneous cross-linking of Ig receptors. If Ig receptors are unoccupied, the interaction results in B-cell apoptotic death. Important roles for the CD40-154 interaction have been described in primary and secondary immune responses to thymus-dependent antigens, memory B-cell development, and in the formation and maintenance of germinal centers (GC).^{41, 140}

B-cell Ig class switching in humoral immune responses is regulated by several signals from T cells.¹⁰⁸ The sequence of intercellular contacts

depicted in Figure 1 leads to T-cell cytokine production. In switching from IgM/IgD production to IgE or IgG4, IL-4 binds to its receptor on the B cell and activates transcription of the ϵ and $\gamma 4$ C_H genes.⁶² This signals an "opening up" of the DNA in the region and its accessibility to molecules capable of recognizing signal sequences and/or modifying DNA. CD40 binding may promote class switching either by inducing production of "switch factors" (DNA-binding or modifying enzymes), by permitting access of preformed factors to the nucleus or a specific nuclear compartment, or both.

CD40 is also expressed on a variety of professional antigen presenting cells (APC) such as dendritic cells, monocytes, and macrophages.^{3, 19} L cells transfected with CD154 can induce expression of CD80 and CD86 and production of IL-8, macrophage inflammatory protein (MIP)-1 α , TNF, and IL-12 in Langerhans' cells.^{20, 21} CD40 cross-linking on monocytes brings about expression of CD86 and HLA-DR.⁸¹ Additional inflammatory signals regulated (at least partially) by CD40 signaling

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Figure 1. Collaboration of T and B cells in the process of isotype switching from the synthesis of IgM to allergen-specific IgE antibody. A B cell presents antigen (allergen) peptides on its surface in association with major histocompatibility complex (MHC) class II molecules. The T-cell receptor (TCR) engages the complex, and adhesion molecules expressed on both cells (not shown) strengthen cellular interaction. T cells then express CD40 ligand (CD154) which binds to B cell CD40. The B cell, in turn, up-regulates expression of B7 molecules (e.g., CD80 or B7-1) which interact with T cell CD28. The T cell responds with production of various cytokines such as interleukin-4 (IL-4). The combination of CD40 and IL-4 signals induces the B cell to undergo the isotype switch to IgE. See article text for additional details. (From Geha RS, Rosen FS: The genetic basis of immunoglobulin-class switching. *N Engl J Med* 330:1008, 1994; Copyright © 1994 Massachusetts Medical Society. All rights reserved.)

include IL-1, IL-6, IL-12, and nitric oxide.^{113, 132, 135, 138} CD40 may also inhibit monocyte apoptotic cell death.¹¹³

The process of CD4⁺ T-cell priming by APC is a dialogue in which both T cell and APC are activated from a resting state to express effector functions. CD40 and CD154 play similar roles in this interaction as described for T cell–B cell cooperation⁵⁷ (see Fig. 1). After T-cell receptor TCR/MHC-peptide recognition, CD154 is expressed and binds to APC CD40. CD80 is induced on the APC and ligates T cell CD28. Intracellular signaling pathways are activated, permitting the T cell to alter its cytokine production and surface molecule expression, thus becoming an effector helper T cell. Simultaneously, the APC is armed to produce a variety of proinflammatory cytokines and other mediators that may influence surrounding cells. The CD40–CD154 interaction also has a role in cross-priming of CD8⁺ T cells. Interaction of CD4⁺ T cells with APC endows them with the ability to become activators for cytotoxic cells.¹³

The broad cellular distribution of CD40 and CD154 (see Table 1) indicates that they may participate in many more aspects of immune regulation. For example, CD154 expressed on activated eosinophils and basophils is capable of eliciting IgE production by B cells in the presence of IL-4.^{46, 47} IL-2 activated natural killer (NK) cells express CD154, and its contact with CD40 may regulate NK cell target recognition, activation of cytotoxicity, and immunoregulatory functions.¹⁷ Cross-linking CD40 expressed by endothelial cells leads to their expression of CD62E (E-selectin), CD106, and CD54.⁷⁵ Thus, CD40–CD154 interactions modulate the activities of diverse participants in immune responses and their exit from the circulation as well. Finally, CD40 is expressed in the thymus on cortical and medullary epithelial cells, interdigitating cells, and B cells.⁴⁴ Thus, CD40 and CD154 may have roles in thymic T-cell selection and formation of the T-cell repertoire.

In this discussion, the authors have merely scratched the surface of the complexity of the CD40–CD154 relationship in the immune system. For further details, the reader is referred to recent excellent reviews.^{40, 58, 141}

PATHOLOGY OF THE CD40–CD154 SYSTEM

CD154 Deficiency

Genetic defects of the *CD154* gene were first described in 1993.^{4, 9, 32, 42, 82} Patients having these mutations are afflicted with a disorder that has been referred to most often as X-HIM. In the most recent World Health Organization (WHO) classification,¹²¹ this disorder is grouped with the combined immune deficiencies, although it is still sometimes included within the humoral deficiencies because of the prominence of hypogammaglobulinemia in its phenotype. Its name derives from its most characteristic immunologic feature: abnormally low levels of IgG, IgA, and IgE associated with normal or elevated levels of IgM. This is

readily understood given the role of CD40–CD154 in the Ig isotype switch described previously. Additional features of this syndrome, such as a susceptibility to opportunistic infections, also are easily understood in light of the important role of CD40–CD154 contact in the T cell–APC interchange.

Clinical and laboratory criteria for diagnosis of X-HIM have recently been published (see box).²⁷

X-HIM: Clinical and Laboratory Diagnosis

Definitive

Male patient with serum IgG concentration at least 2 standard deviations (SD) below normal for age and one of the following:

1. Mutation in the *CD40L* gene
2. Maternal cousins, uncles, or nephews with confirmed diagnosis of X-HIM

Probable

Male patient with serum IgG concentration at least 2 SD below normal for age and all of the following:

1. Normal numbers of T cells and normal T-cell proliferation to mitogens
2. Normal or elevated numbers of B cells but no antigen-specific IgG antibody
3. One or more of the following infections or complications
 - a. Recurrent bacterial infections in the first year of life
 - b. *Pneumocystis carinii* infection in the first year of life
 - c. Neutropenia
 - d. Cryptosporidium-related diarrhea
 - e. Sclerosing cholangitis
 - f. Parvovirus-induced aplastic anemia
4. Absent CD40 ligand cell surface staining on activated CD4⁺ T cells as assessed by binding to soluble CD40 or by binding of monoclonal antibody to CD40 ligand

Possible

Male patient with serum IgG concentration at least 2 SD below normal for age, normal numbers of T cells and B cells, and one or more of the following:

1. Serum IgM concentrations at least 2 SD above normal for age
2. *P. carinii* infection in the first year of life
3. Parvovirus-induced aplastic anemia
4. Cryptosporidium-related diarrhea
5. Severe liver disease (sclerosing cholangitis)

Since 1961, approximately 200 separate cases fulfilling the criteria for definite or probable diagnosis of CD154 deficiency have been reported in the literature. The single largest case series is that of Levy et al,⁸⁷

containing 56 patients. Forty-eight of these had defined CD154 mutations; the other 8 had pedigrees with unmistakable X-linked inheritance ("possible" X-HIM by the criteria described earlier). This study conducted the most thorough investigations of a large cohort of patients reported to date. This series contained 22 patients for whom some clinical data were contained in previous case reports. None of these patients was included in the authors' separate analysis. The second largest series, containing 45 patients with defined CD154 mutations, was reported by Seyama et al¹²⁹; however, individual patient clinical data were not included. Thirty-seven of these patients are included in the authors' analysis based on earlier case reports.^{4, 9, 65, 68, 70, 98, 107, 123, 127} The authors studied 71 additional cases (total 108) in which a CD154 gene defect or lack of CD154 expression was documented. For 69 of these 108 cases, some clinical information was suitable for inclusion in the authors' analysis.*

Clinical Features of CD154 Deficiency

Clinical data from the cases the authors reviewed are summarized in Table 2, where they are compared with those presented by Levy et al.⁸⁷ Not all reported cases were evaluable for all of the clinical features listed.

Of 29 case reports containing the age at presentation, the distribution was heavily skewed toward the first year of life, with 66% of patients identified before their first birthday. The median age at presentation was 8 months; 90% of patients presented by 3 years of age. Only three cases were reported of previously healthy children diagnosed at 8, 14, and 17 years of age when presenting with their first clinical manifestations of X-HIM, parvovirus-induced anemia, or pancytopenia.¹²⁸ Levy et al⁸⁷ reported a mean age at presentation of 12.9 months.

The predominant clinical manifestations in both groups of patients included recurrent viral and bacterial infections of the upper and lower respiratory tract in addition to interstitial lung disease most often caused by *P. carinii*; neutropenia, which may be chronic, episodic, or cyclic; chronic or recurrent diarrhea with failure to thrive; oral or perirectal ulcerations; liver disease (see forthcoming discussion); and a variety of additional bacterial and other infections (see Table 2). Although parvovirus-associated anemia had been noted in past case reports,¹²⁸ it may have been an underdiagnosed complication of X-HIM before the report of Levy et al.⁸⁷ A seronegative polyarthritis has been described in a few case reports of X-HIM.^{90, 116, 146} Levy et al⁸⁷ found polyarthritis in 11% of their patients.

Reported malignancies included two cases of hepatocellular carcinoma and a primitive neuroectodermal tumor in the series of Levy et al,⁸⁷ one lymphoma,⁹ and one malignant carcinoid⁷² in the previous case

*References 2, 6, 28, 29, 31, 32, 37, 38, 42, 51, 63, 69, 72, 77, 80, 82, 85, 86, 90, 94-96, 106, 116, 125, 128, 129, 131, 136, 137, 139, 145, and 146.

Table 2. CLINICAL FEATURES OF 69 CASE REPORTS OF DEFINITE OR PROBABLE CD154 DEFICIENCY

	Reviewed Cases	Levy et al ⁸⁷
Median age at presentation	8 mo (n = 29)	—
Respiratory tract		
Recurrent viral and bacterial URTI, LRTI*	74%	88%
Interstitial lung disease, PCP	34%	39%
Blood		
Neutropenia*	65%	68%
Anemia (parvovirus)*	6%	32%
Gastrointestinal tract		
Diarrhea, failure to thrive*	17%	55%
Stomatitis*	17%	45%
Perirectal ulcers/fissures*	6%	—
Candidiasis, oral or mucocutaneous*	9%	—
Hepatic disease (sclerosing cholangitis, <i>C. parvum</i> , HBV, HCV infection)	8%	10–75% (age dependent)*
Other infections		
Sepsis	4%	14%
Meningoencephalitis	9%	8%
Cryptococcosis, disseminated or CNS*	9%	2%
Toxoplasmosis, disseminated or CNS	6%	2%
Cellulitis, osteomyelitis	4%	—
Seronegative arthritis	8%	11%
Malignancy	4%	4%
Lymphadenopathy	1%	13%
Findings noted only in one or a few case reports	Additional features noted in Levy et al	
Infections		
Recurrent herpes simplex or varicella-zoster	Disseminated CMV	
Focal or disseminated disease with Bacille Calmette-Guerin (BCG)†	Adenoviral pneumonia	
Disseminated <i>Histoplasma capsulatum</i>	Atypical mycobacterial pneumonia	
Fungal meningitis	Giardiasis	
Pyelonephritis	Salmonellosis	
Other manifestations	<i>Entamoeba histolytica</i>	
Pancytopenia	Inflammatory bowel disease	
Ataxia	Degenerative encephalopathy	

*Feature noted as part of the clinical presentation.

†Feature also noted in the series of Levy et al.⁸⁷URT I = upper respiratory tract infection; LRTI = lower respiratory tract infection; PCP = *P. carinii* pneumonia; HBV = hepatitis B virus; HCV = hepatitis C virus; CMV = cytomegalovirus.

reports. In addition, a wide variety of infectious and other conditions were limited to one or a very few patients in both groups (see Table 2).

Hepatic disease in CD154 deficiency is worthy of separate discussion and has been analyzed in greatest detail in the report of 53 patients of Hayward et al⁶⁵ (17 with CD154 mutations, 21 with absent CD154 expression, and 15 with X-linked pedigrees). Overall, 50% of patients had elevated transaminases, some with abnormalities on liver biopsy, at some point in their clinical course. The prevalence of liver disease was observed to increase with time of survival. Only 10% of patients alive at

5 years of age were affected, whereas 75% of patients alive at 20 years had liver disease. Fourteen patients had infection with *Cryptosporidium parvum*; cytomegalovirus (CMV) was identified in three. Sclerosing cholangitis and/or cirrhosis were identified in 8 of 9 patients who eventually developed a variety of hepatic or gastrointestinal malignancies. Seyama et al¹²⁹ also reported 4 of 45 patients with sclerosing cholangitis all due to *C. parvum*. They also noted five gastrointestinal tumors. Hepatic disease was reported in 6% of the cases the authors reviewed. These included sclerosing cholangitis and hepatitis with cause unspecified, or in association with hepatitis B or C virus infection in a few cases. It is only recently that the importance of *C. parvum* for hepatic disease in X-HIM has been appreciated.

The ages and causes of death among previously reported cases of definite or probable X-HIM are listed in Table 3. Additional diagnoses leading to death in the series of Levy et al⁸⁷ included liver disease (before or after liver transplantation), peritonitis, CMV encephalitis, CMV infection after bone marrow transplantation, and a primitive neuroectodermal tumor of the colon. The age at death in that group ranged from 9 months to 23 years. A survival curve based on the data of Levy et al⁸⁷ is shown in Figure 2. Survival decreases slowly initially, with approximately 10% dying before age 12 years. Subsequently, mortality accelerates, with roughly 6% dying per year to age 25 when only 15% to 20% of patients are alive.

Immunologic Features of CD154 Deficiency

Serum Ig levels in past case reports in comparison to the data of Levy et al⁸⁷ are shown in Table 4. Five of 48 patients (10%) with IgG reported before institution of replacement therapy had a level greater than 4 g/L. Only 1 had a level above 6 g/L (6.5), which later decreased to 2.8.⁸⁵ The highest IgG level reported by Levy et al⁸⁷ was 2.64 g/L. In past reports of 43 patients, 58% had no IgA detected at diagnosis; however, 5 patients (10%) had IgA greater than 0.7 g/L. For some patients for whom more than one Ig measurement was reported, the level of IgG or IgA rose over time.^{2, 10, 86} In at least 1 patient, IgA rose to

Table 3. CAUSES OF DEATH REPORTED AMONG 69 CASES OF CD154 DEFICIENCY

Age at Death	Cause of Death
6 mo	Protracted diarrhea
7 mo	<i>P. carinii</i> pneumonia*
3 y	Echovirus meningoencephalitis
6 y	Disseminated <i>C. neoformans</i>
6 y	Lymphoma, type not specified
7 y	Fungal meningitis
9 y	Disseminated toxoplasmosis
?	Peritonitis*

*Noted as a cause of death in the series of Levy et al.⁸⁷

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Figure 2. Kaplan-Meier survival curve resulting from a retrospective analysis of 56 patients with X-linked hyper-IgM syndrome. N=number. (From Levy J, Espanol-Boren T, Thomas C, et al: Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 131:47, 1997; with permission.)

a high level (6.5 g/L) later in the clinical course.¹⁰ Levy et al⁸⁷ also observed three patients (5%) with elevated IgA.

Four of 51 patients had IgM levels higher than those observed in the series of Levy et al, ranging from 15 to 20 g/L. Overall, 61% of 51 reviewed cases had IgM less than 3 g/L at diagnosis. Levy et al⁸⁷ reported normal IgM levels in 53% of their series. In previous reports where repeated measurements were recorded, IgM tended to increase with age,² although this was not always true. Levy et al⁸⁷ also noted a trend of increasing IgM level with age. In past reports, and in the series of Levy et al, serum IgE levels tended to be low.

Impaired vaccine responses were noted in six of the cases the authors reviewed. These are not reported in Levy et al,⁸⁷ but this information is contained in a data base of CD154 mutations (see later discussion) in which impaired response is noted in a majority of patients. Impaired primary and secondary responses to the model neoantigen

Table 4. SERUM IMMUNOGLOBULIN LEVELS IN PATIENTS WITH CD154 DEFICIENCY

	Previous Case Reports					Levy et al ⁸⁷	
	Mean	SD	n	Median	Range	Mean	Range
IgG (g/L)	1.58	1.71	48	0.92	0–6.5	1.1	0–2.64
IgA (g/L)	0.17	0.31	48	0	0–1.23	0.2	0–2.92
IgM (g/L)	3.88	4.77	51	1.70	0.2–20	2.62	0.3–13

bacteriophage X174 has been clearly demonstrated in nine patients studied by Seyama et al.¹²⁹ Isohemagglutinin levels have been normal in all patients for whom this information is reported.

CD40 crosslinking in the presence of IL-4 is a highly effective system for driving selective IgE production.^{4, 9, 42, 82} Cell lines transfected with CD154 bearing mutations found in patients with X-HIM fail to induce B cells to produce IgE in the presence of IL-4, demonstrating its crucial role in T-cell-B cell interaction in the production of isotypes other than IgM.⁴ On the other hand, B cells from X-HIM patients proliferate and produce IgG, IgA, and IgE after a variety of stimuli. Ligation of CD40 with monoclonal antibodies or soluble CD154 together with additional stimuli such as anti-IgM antibodies, cells expressing IgG Fc receptors, IL-2, IL-10, or *Staphylococcus aureus* Cowan strain, leads to synthesis of IgM, IgG, and IgA.^{2, 35, 82, 123, 124} Production of IgG and IgA is normal in some, but not all, of these studies. The discrepancies may be due to differences in the specific stimuli used and in heterogeneity among X-HIM patients. It is possible that B-cell development in the absence of functional CD154 alters the regulation of class-switching within B cells in subtle ways. This question remains to be answered conclusively.

Numerical lymphocyte phenotyping data are relatively scarce in many previous case reports of X-HIM. In 15 cases for which some data are available, only 2 reported inversion of the CD4/CD8 ratio; B-cell percentages were normal in all cases. Levy et al⁸⁷ noted abnormally low numbers of B- or T-lymphocyte subpopulations in less than 10% of their patients.⁸⁷ Eleven of 12 previously reported patients with definite or probable X-HIM and 93% of the 46 tested in the series of Levy et al⁸⁷ had normal T-cell proliferative responses to phytohemagglutinin (PHA) in vitro. All of four previously reported patients were noted to have normal in vitro T-cell response to tetanus toxoid. Ameratunga et al⁵ reported a 50% to 80% rate of defective response to specific antigen in vitro among five patients, although response was detected in a few patients after booster immunization. Levy et al⁸⁷ reported reduced T-cell proliferation with tetanus toxoid in 6 of 16 (38%) patients tested. Normal skin delayed hypersensitivity responses have been reported in a few patients with X-HIM.^{70, 86, 125} One patient has been described with absent CD56⁺/16⁺ NK cells and absence of spontaneous cytotoxicity against K562 cells.¹⁰⁶

Jain et al⁷² observed clear reductions in the percentages of circulating T cells bearing CD45RO in patients with X-HIM, suggesting a reduction in antigen priming. They also showed that CD154⁻ T cells have greatly reduced production of interferon (IFN)- γ after stimulation via the TCR-CD3 complex or by polyclonal mitogen (PHA). This led to reduced IL-12 and TNF production by APC. Subauste et al¹³⁶ reported similar findings using T cells from X-HIM patients chronically infected with *Toxoplasma gondii*.

Some studies have shown that lymph nodes in patients with X-HIM contain primary but not secondary lymphoid follicles⁸⁹; however, CD154 mutations were not demonstrated in any of these patients. Facchetti et

al³⁷ studied lymph nodes and mucosal lymphoid tissues from two CD154 X-HIM patients. They similarly found a lack of germinal centers and further observed that while paracortical T-cell areas developed in abortive germinal centers, follicular dendritic cells were strikingly depleted. In one patient with high serum IgM, these authors also found many IgM-secreting plasma cells in the lamina propria of the colon. They suggested that chronic antigen stimulation might lead to the high levels of IgM characteristic of many X-HIM patients. It is possible that reductions in IgM after IVIG therapy seen in some patients⁸⁷ may be due to lower levels of antigenic stimulation after improved microbial clearance.

Agematsu et al² showed that patients with X-HIM lack IgD⁺ memory B cells, which is consistent with the abnormal development of germinal centers, the primary site for B memory cell development.⁹² Chu et al²³ found that somatic mutation in heavy chain variable region genes was reduced to levels of approximately 25% to 50% of normal in B cells from X-HIM patients,²³ whereas Razanajaona et al¹¹⁶ detected no mutations in two patients and 70% of normal levels in another. The latter patient had weak T-cell expression of mutant CD154 that was capable of partial agonist activity, enough to drive mutation but not normal isotype switching. These findings are also consistent with abnormal B-cell development in germinal centers, where the majority of Ig variable gene somatic hypermutation occurs.⁹²

Although several of the clinical characteristics of X-HIM may be associated with autoimmunity, serum autoantibodies have been found in only a minority of cases.⁸⁷ Interestingly, Lacroix-Desmazes et al⁸³ found significant reductions in the spectrum of autoantigens bound by serum IgM in patients with X-HIM. In addition, autoreactivity was almost absent from serum IgG. Their data suggest that CD154 may be required for establishment of the autoreactive B-cell repertoire, at least for a significant fraction of autoantigens.

Many of the immunologic aspects of CD154 deficiency are recapitulated in mice after targeted disruption of this gene.^{16, 59, 118, 149} These mice have normal peripheral T-cell numbers and mitogen responses, although the proportion of CD4⁺/CD8⁺ double-positive T cells in the thymus is somewhat reduced. B-cell numbers are normal, IgG and IgE are reduced, and IgA falls over time. IgM is normal if mice are housed in germ-free facilities. Secondary lymphoid tissues are devoid of fully formed germinal centers, although the paracortical T-cell areas appear to develop, as they do in lymph nodes of patients with X-HIM.³⁷ There is no antibody response to T-dependent antigens; responses to T-independent antigens are normal. These mice have been shown to have defective T-cell priming during immunization and have impaired defense against leishmania. Cytotoxic activity of CD154-deficient murine CD8⁺ T cells is normal against a variety of viruses⁵⁶; however, cytotoxic T-cell memory appears to be impaired. Disruption of CD40 signaling in mice using an antibody blocking CD154 led to impaired clearance of *P. carinii*.¹⁴⁷

Many recent studies have investigated the responses of CD154-

deficient mice against a variety of infectious organisms. Increased susceptibility to infection is not universal, although the nature of the immune response is clearly different for many. These differences in susceptibility are doubtless due to the many different mechanisms of induction and execution of immune responses in each particular situation, the manner in which different organisms infect the host, and the strategies each has evolved to evade host defenses. This area has been the subject of an excellent recent review.⁵⁶

Female Carriers of CD154 Defects

Obligate female carriers of X-HIM have not been noted to suffer from an increased rate of infections and do not display the abnormal serum Ig profile characteristic of affected males.⁶⁸ CD154 expression on stimulated peripheral blood mononuclear cells from four female carriers ranged from normal (about 70% of cells) to as low as 12%. Similarly, CD154 expression on activated purified CD4⁺ T cells ranged from 33% to 68% of cells in comparison to 93% in the control. DNA analysis confirmed random inactivation of the X chromosome in carrier T cells.⁶⁸ These data suggest that lack of functional CD154 does not preclude T-cell exit from the thymus, and that the presence of as little as one fifth to one third the normal fraction of CD154⁺ T cells permits normal cellular immunity.

One female patient with clinical features of X-HIM has been reported due to apparent extreme nonrandom X-inactivation.²⁹ The authors suggest that some female HIM carriers with extreme nonrandom lyonization may express an HIM phenotype, and may be misdiagnosed as having an autosomal recessive form of HIM (see forthcoming discussion) or common variable immune deficiency. Similar situations have been encountered in female carriers of chronic granulomatous disease⁷³ and Wiskott-Aldrich syndrome.¹¹⁰

Genetics of CD154 Deficiency

Levy et al.⁸⁷ observed that 43% of their cohort of patients did not have a family history of disease. This agrees fairly well with the estimated 50% occurrence of novel mutations that is expected for gene defects on the X-chromosome.¹¹⁴ In 60 previously reported patients for whom family history data are available, 27% were sporadic cases, probably caused by selection bias before the discovery of CD154 mutations in X-HIM. X-HIM occurs in all racial groups.¹²⁹

The structure of the CD154 mRNA is shown in Figure 3. Also shown is a chart displaying the relative proportions of various types of mutations identified in the CD154 gene. These data are derived in part from a data base of CD154 mutations, which is accessible through the World Wide Web¹⁰⁰ (see Figure 3 legend). The data base contains 65 entries, most from patients in the series reported by Levy et al.⁸⁷ Although missense mutations predominate, all potential forms of gene disruption are observed. The mutations cluster most heavily in the

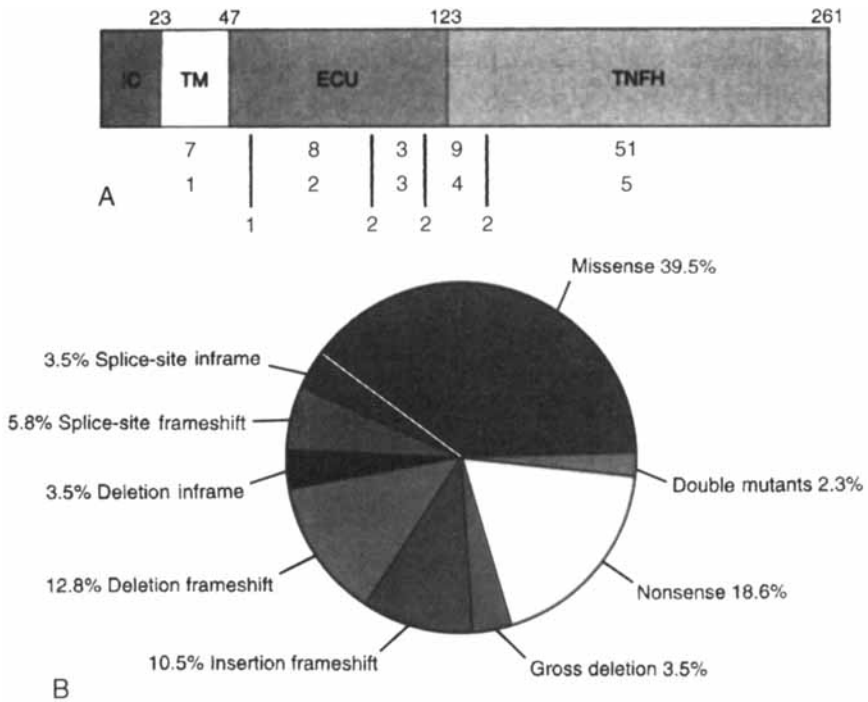


Figure 3. *A*, *CD40L* structure and mutation distribution. Vertical bars below the figure indicate the exon boundaries. The numbers in the top row represent the number of families with mutations in the respective exons, which are numbered in the second row. The sum of families having splice-site mutations is shown below the vertical bars. *B*, Distribution of the type of mutations in the *CD40L* gene identified in families with X-linked hyper-IgM (X-HIM) syndrome. The database can be accessed on the World Wide Web at <http://www.expasy.ch/cd40lbase> or at <ftp://expasy.hcuge.ch/database/cd40lbase>. ECU = extracellular unique; IC = intracytoplasmic; TM = transmembrane; TNFH = tumor necrosis factor homology. (From Notarangelo LD, Parolini O, Albertini A, et al: *CD40Lbase: A database of CD40L gene mutations causing X-linked hyper-IgM syndrome*. Immunol Today 17:511, 1996; with permission.)

membrane distal TNF homology region. The tryptophan at codon 140 appeared as the most prominent mutational "hot spot" with mutations in eight families.

Genotype-phenotype correlations are not yet completely clear, according to this compilation. Previous studies analyzing the expression of CD154 on activated T cells from patients suggested that disease was milder where surface expression and binding of soluble CD40-ligand were greatest¹²⁹ (see below).

Diagnosis of CD154 Deficiency

The clinical features of X-HIM are not completely distinct, and there is significant overlap with common variable immune deficiency, some

syndromes of severe combined immune deficiency, transient hypogammaglobulinemia of infancy, and possibly, atypical forms of Bruton's or X-linked agammaglobulinemia. The diagnosis of X-HIM most often comes to mind in a patient with recurrent respiratory tract infections, with or without neutropenia, with or without *P. carinii* pneumonia. The finding of low IgG and IgA with normal or elevated IgM is highly suggestive but not diagnostic. In X-HIM, the T- and B-cell numbers are most often normal, with normal mitogen proliferation, and possibly an impaired T-cell antigen response *in vitro*.

The diagnosis of probable or definite X-HIM rests on the determination of surface expression of CD154 on activated T cells and characterization of the *CD154* gene. In most studies, peripheral blood mononuclear cells (PBMC) or purified T cells are examined. Convenient methods of T cell activation and flow cytometry analysis of CD154 expression using whole blood also have been developed.¹⁰¹ Seyama et al¹²⁹ studied activated T cells from 45 X-HIM patients by flow cytometry with labeled polyclonal anti-CD154, four different anti-CD154 monoclonal antibodies (mAbs), and a CD40-Ig constant region fusion protein. The sequences of the coding regions of the *CD154* gene in all of these patients were also determined. These authors defined five distinct patterns of cell surface staining based on the pattern of reaction with these reagents. They noted that T cells from five of these patients bound the CD40-Ig fusion protein normally. Thus, normal staining of CD154 on activated T cells, either with antisera, mAbs, or soluble CD40, cannot exclude the diagnosis of X-HIM in approximately 10% of patients. When suspicion is high, and CD154 expression is normal, genetic analysis is required.

For determining a specific mutation, polymerase chain reaction (PCR) amplification, cloning, and sequencing is the most convenient and direct method. Once the mutation is known, less labor intensive methods such as PCR with sequence-specific oligonucleotide primers (PCR-SSOP), exon-specific PCR amplification and sequence specific conformation polymorphism (PCR-SSCP) analysis, or hybridization with labeled oligonucleotide primers may be used for screening potential carriers. These methods also may be used in prenatal diagnosis of potentially affected embryos.^{78, 88, 127, 143} Linkage analysis of polymorphic dinucleotide repeats within the *CD154* gene also has been used in prenatal diagnosis of X-HIM.³³

Therapy of CD154 Deficiency

Intravenous immunoglobulin (IVIG) replacement therapy is indicated in the majority of X-HIM. Levy et al⁸⁷ documented a clear benefit of IVIG in 90% of their series of patients who received regular infusions of IVIG (51/56 patients). The same was true for 89% of the IVIG-treated patients (42/45) in the series of Seyama et al.¹²⁹ Dosage regimens are standard as in any syndrome of hypogammaglobulinemia (300–600 mg/kg every 3 to 4 weeks). Some patients with mild disease do not require regular IVIG therapy and may not even suffer from recurrent bacterial infections. This was the case in 5 of the 56 patients of Levy et al⁸⁷ and

in 3 of the 45 patients of Seyama et al.¹²⁹ Antibiotic prophylaxis also has been applied for the prevention of recurrent infections of the respiratory tract.

Prophylaxis of *P. carinii* pneumonia with trimethoprim-sulfamethoxazole is also indicated in all cases of X-HIM. This was successful in preventing *P. carinii* pneumonia in 96% of the patients so treated in the series of Levy et al.⁸⁷ Recombinant human granulocyte colony-stimulating factor (G-CSF) has been used in the treatment of neutropenia associated with X-HIM with mixed success.^{6, 9, 106, 145} Levy et al⁸⁷ observed normalization of the neutrophil count in 73% of their G-CSF-treated patients.⁸⁷ They also observed that IVIG therapy alone led to resolution of neutropenia in 14 of 29 (48%) patients.

Hayward et al⁶⁵ reported a relapse of sclerosing cholangitis in three patients who underwent liver transplantation. Martinez-Ibanez et al⁹⁴ reported three patients with X-HIM who received liver transplants at 6 to 14 years of age for liver failure associated with sclerosing cholangitis. All three developed relapses that led to death in two. Cryptosporidium and leishmania were identified in the native liver of one patient who died after transplantation.

Successful bone marrow transplantation has been reported in detail in five patients with X-HIM. In four of these cases, the donors used have been HLA-matched siblings.^{77, 80, 125, 137} Pretransplant conditioning was applied in all cases. Only one patient did not receive prophylactic immunosuppression for graft-versus-host disease (GVHD).⁷⁷ GVHD did not develop in this patient and did not progress beyond grade I in one other patient.¹²⁵ One patient has received a cadaveric liver followed by bone marrow from a 10 HLA-antigen matched unrelated donor with subsequent GVHD prophylaxis with success.⁶⁰ In the patients of Levy et al,⁸⁷ two HLA-matched sibling and one unrelated donor bone marrow transplants were recorded. There was one death from CMV infection after transplantation. Seyama et al¹²⁹ noted one bone marrow transplantation without further data.

As discussed, some mutant CD154 proteins are expressed on the cell surface and are capable, in some cases, of interacting with CD40.¹²⁹ It has been further demonstrated that mutant and normal CD154 molecules expressed in the same cell may form heterotrimers with partial agonist activity.^{45, 130} This must be taken into consideration in designing gene therapy for X-HIM.

Autosomal Recessive HIM

Clinical Features

The authors reviewed reports of 52 patients with a form of HIM having autosomal recessive inheritance (ARHIM).^{*} In 38 of these patients, the pedigree was clearly consistent with autosomal recessive inheritance.

^{*}References 7, 11, 14, 15, 26, 34, 43, 48–50, 66, 71, 83, 93, 97, 103–105, 111, 120, 133, and 134.

Table 5. GENDER AND FAMILY HISTORY IN PATIENTS WITH ARHIM

Gender		Family History	
Male	25 (48%)	Positive	16 (43%)
Female	27 (52%)	Negative	21 (57%)
Total	52		37

Of the remaining 14 patients, 8 are female, and 3 of the remaining 6 males had a normal *CD154* gene sequence, and normal *CD154* expression on activated T cells in all 6 males. The data regarding inheritance in this group are shown in Table 5. Data regarding family history were not clear for all patients. Consanguinity was noted in four kindreds.

The clinical features of ARHIM are distinct from those of X-HIM. The features include the following:

- Recurrent viral and bacterial respiratory tract infections* (83%)
- Diarrhea, failure to thrive (*Cryptosporidium* in one) (17%)
- Massive lymphadenopathy (8%)
- Short stature (two kindreds, growth hormone deficiency in one) (8%)
- Bacterial skin infections (8%)
- Features noted in two patients each
 - Enteroviral meningoencephalitis*
 - Chronic hepatitis C infection
 - Meningococcal meningitis*
 - Coombs' positive hemolytic anemia
 - Immune thrombocytopenic purpura
 - Systemic and discoid lupus erythematosus (one each)
 - Hypohidrotic ectodermal dysplasia (one kindred)†
 - Hodgkin's lymphoma (one nodular sclerosing, one mixed cellularity)
- Features noted in only one patient
 - Neutropenia†
 - Infections with "unusual organisms" not specified†
 - Severe varicella infection
 - Sepsis (*Staphylococcus aureus*)*
 - Aspergillus pneumonia
 - Mucosal candidiasis
 - Giardiasis
 - Necrotizing lymphadenitis
 - Hepatitis, unspecified cause
 - Seronegative rheumatoid arthritis†
 - Neurodegenerative disease of unknown cause*

*Noted as a cause of death.

†Features isolated to patients with demonstrated defects of *CD40* signaling.

Most notable are the low incidence of opportunistic infections, neutropenia, and hepatic disease (apart from hepatitis C infection), which were noted in only one patient each. The features of ARHIM appear to

be more consistent with the expression of hypogammaglobulinemia, without severe deficits in cell-mediated immunity. The clinical data presented in these case reports permit no conclusions comparing ARHIM to X-HIM with respect to the onset of disease or survival. Some of the causes of death in these patients have been noted (see earlier discussion).

Sixteen patients reported with ARHIM have been demonstrated to have defective signaling via CD40, although surface expression was normal.^{26, 34, 133} The sequence of the CD40 gene was determined for three patients and was normal.³⁴ The clinical features of this subset of patients do not appear to be distinct from patients whose CD40 function has not been investigated. In one report, the *CD154* coding sequence was determined for 17 patients with the HIM phenotype.²⁶ In all of the 4 patients with normal *CD154* genes, defective CD40 function was documented. This suggests that this impairment of CD40 function may occur in a majority of ARHIM patients, although this requires further study. Three female patients with ARHIM have been described with normal CD154 expression and normal signaling via CD40.^{7, 105}

Immunologic Features of ARHIM

The serum concentrations of IgG and IgA in ARHIM do not differ from those noted in X-HIM. One patient with defective CD40 signaling had normal total IgG (8.5 g/L) but had absent IgG2 and IgG4.³⁴ There is a trend toward higher levels of IgM in ARHIM with mean 8.5 g/L, SD 8.5, and median 6.4 (n = 40) (compare with values for X-HIM in Table 4). Four of 52 patients had IgM greater than 20 g/L. The absence of specific antibody responses was noted in one patient and normal isohemagglutinins in three. B-cell percentages were noted to be normal in 18 (15 with defective CD40 signaling) and low in 2 (CD40 signaling defect not known). One study documented only IgM production in vitro in response to pokeweed mitogen.¹⁴ Normal germinal centers (GC) were found in the lymphoid tissue of two patients not studied with respect to CD40,^{71, 93} whereas absence of GC in the spleens of two patients with CD40 signaling defects was observed.³⁴

Among the 36 patients with unknown CD40 functional status, five were reported to have normal T-cell subsets, and five had inversion of the CD4/CD8 ratio. T-cell mitogen responses were normal in six patients and low in two. T-cell responses to antigens in vitro were not reported. Cutaneous delayed hypersensitivity was normal in six and low in one. Among the 16 patients with defective CD40 signaling, all of 10 patients in one series had normal T-cell subsets and mitogen and antigen proliferation.³⁴

Revy et al¹¹⁹ studied four previously reported patients with defects of CD40 signaling in B cells³⁴ and found that monocytes and dendritic cells responded normally to crosslinking of surface CD40. This suggests that the defect in this subset of patients may be restricted to B cells and may explain the relative lack of opportunistic infections in these patients. It is not yet known whether the same genetic defect leads to impairment of CD40 signaling in all of these patients. One group analyzed the intracellular events after CD40 ligation in B-cell lines from patients who

had defective CD40 signaling.³⁴ They found no activation of phosphoinositol 3' kinase in five lines analyzed. Induction of the transcription factors nuclear factor κ B (NF- κ B) and activator protein-1 (AP-1) proceeded normally in four and not in another, suggesting genetic heterogeneity in defective CD40 signaling. Finally, cytoplasmic signal transducing molecules were studied in several patients. The CD40 binding protein (CD40bp) mRNA was normally expressed, its coding region sequence was normal in three patients. In one patient with defective induction of NF- κ B, the coding region sequence of the TNFR associated factor 2 (TRAF2) was normal. An analysis of CD40 signaling is beyond the scope of this discussion; the reader is referred to an excellent recent review by van Kooten and Banchereau.¹⁴¹

Mice rendered defective in CD40 by targeted gene disruption have a phenotype similar to mice lacking CD154.^{18, 74, 79} This is not altogether surprising, and CD40 deficiency probably does not constitute a model analogous to ARHIM. Even in patients with defective CD40 signaling, the data of Revy et al¹¹⁹ suggest that the B-cell lineage may be more affected than other cell types. This clearly is not the case for CD40-deficient mice.

Patients with Possible X-HIM

Sixty-four additional case reports in the literature cannot definitely be assigned to either X-HIM or ARHIM. This is either because they have a family history consistent with X-linked inheritance without mutations described in the kindred (26 patients) or because the cases are sporadic. The clinical features of 36 of these cases are such that they are very likely to be X-HIM. The only findings somewhat distinct from those just described include two male patients with hundreds of common warts^{22, 150} (one sporadic, one with a positive family history) and progressive multifocal leukoencephalopathy due to papovavirus in one sporadic male case.¹¹⁷

SUMMARY

The discovery of *CD154* gene defects in X-HIM brought into focus the critical role played by CD40-CD154 interactions in diverse areas of immune function. This led quickly to many striking observations that may soon yield great benefits in controlling immune responses in the contexts of infectious diseases, autoimmune disorders, and solid organ and bone marrow transplantation.

Blockade of the CD40-CD154 interaction suppresses autoimmunity in several murine models.⁵⁸ This inhibition may result from perturbations of T-cell priming, recruitment into tissues, and execution of effector functions. During islet cell transplantation, pretreatment with allogeneic B cells and anti-CD154 antibodies induces tolerance to islet cells from

the same donor.¹⁰⁹ The administration of anti-CD154 antibodies together with allogeneic T cells can prevent GVHD following bone marrow transplantation.³⁶ Anti-CD154 antibodies also have extended graft survival in murine cardiac allotransplantation.⁶¹ These effects result, at least in part, from perturbing normal collaboration between T cells and APC, preventing both from achieving full effector competency.

Understanding of the role of CD154 in the clinical and immunologic manifestations of X-HIM led immediately to its application in diverse and important fundamental investigations of immune regulation. The knowledge gained by the study of X-HIM exemplifies the potential rich harvest of research stimulated by the analysis of patients with naturally occurring genetic mutations.

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