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X-Linked Lymphoproliferative Disease: Twenty-Five Years after the Discovery

THOMAS A. SEEMAYER, THOMAS G. GROSS, R. MAARTEN EGELER, SAMUEL J. PIRRUCCELLO, JACK R. DAVIS, CHRISTOPHER M. KELLY, MOTOHIKO OKANO, ARPAD LANYI, AND JANOS SUMEGI

Departments of Pathology [T.A.S., T.G.G., S.J.P., J.R.D., C.M.K., A.L., J.S.] and Pediatrics [T.A.S., T.G.G.], University of Nebraska Medical Center, Omaha, Nebraska 68198-3135, Department of Pediatrics [R.M.E.], Sophia Children's Hospital, Rotterdam, The Netherlands, and Department of Pediatrics [M.O.], Hokkaido University, Sapporo, Japan

ABSTRACT

The X-linked lymphoproliferative disease (XLP), one of six described X-linked immunodeficiencies, stems from a mutation at Xq25 which renders males impotent to mount an effective immune response to the ubiquitous EBV. Purtilo, who first observed this disease in 1969, established a Registry in 1980 to serve as a worldwide resource for the diagnosis, treatment, and research of this condition. Since Purtilo's death in late 1992, the Registry and research unit have not only continued to function as a worldwide consultative service, but have contributed the following. First, the number of affected boys has continued to grow; some 272 among 80 kindreds have been identified. Second, some boys (10%) who inherit the mutated XLP gene are immunologically abnormal even before evidence of EBV exposure. Third, the search for the XLP gene has been narrowed to a small region on Xq25. Its identification is near at hand; once cloned, this gene may well illustrate how the body orchestrates the complex immune response to EBV. Therein lies the justification for the quest for this gene, not only for the benefit of the few surviving boys and those to be born to female carriers, but also for defining its role in defending the body against a ubiquitous DNA virus. (***Pediatr Res* 38: 471–478, 1995**)

Abbreviations

EBNA, Epstein-Barr nuclear antigen
IFN, Interferon
IM, infectious mononucleosis
RFLP, restriction fragment length polymorphism
TH1, T-helper type 1
TH2, T-helper type 2
VAHS, virus-associated hemophagocytic syndrome
VCA, viral capsid antigen
XLA, X-linked agammaglobulinemia
XLHM, X-linked hypergammaglobulinemia M
XLP, X-linked lymphoproliferative disease
YAC, yeast artificial chromosome

This review traces the history of relevant events before and after the discovery of a disease of the immune system some 25 y ago. What began as an astute observation at the autopsy table in 1969 is now the subject of intense molecular investigation in several laboratories across two continents. To some extent, the latter quest is an intellectual and technologic assault to disinter a gene, establish its sequence, determine its protein product, and, above all, define its normal function. Through the gifts of parents and friends, the William C. Havens Foundation was established to promote research into the pathogenesis, treatment, and ultimate resolution of XLP.

Correspondence and reprint requests: Thomas A. Seemayer, M.D., FRC Path, Department of Pathology and Microbiology, University of Nebraska Medical Center, 600 South 42nd Street, Omaha NE 68198-3135.

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EVENTS LEADING TO THE RECOGNITION OF XLP

The recognition in 1969 and ultimate description of XLP in 1975 were made possible by a series of findings in the late 1950s, 1960s, and early 1970s which related to the EBV and immune dysfunction. These are briefly summarized.

The story begins with the description by Denis Burkitt in 1958 of an unusual, rapidly growing, and lethal sarcoma in children in Uganda and central Africa (1). Although the tumor featured a distinct predilection for extranodal sites, O'Conor established in 1961 that it was a lymphoma (2); thus, Burkitt's lymphoma entered the literature. In 1964, Epstein and Barr, working in the United Kingdom, reported the successful cultivation of these malignant lymphoblasts (3) and demonstrated virus particles in the tumor (4). The virus, one of seven members of the Herpesvirus family, became known as the Epstein-Barr virus (EBV).

Not long thereafter, the Henle's, working in Philadelphia, made several interesting observations. First, EBV was capable of transforming "*in vitro*" normal peripheral blood lymphocytes into immortalized lymphoblastoid cell lines (5). Second, they confirmed the observation made months previously by Pope (6) that peripheral blood lymphocytes obtained from a patient (one of their technicians) with IM could be cultivated *in vitro*. Moreover, a small percentage of these cells contained EBV antigens, and postinfectious serum contained EBV antibodies, whereas preinfectious serum was devoid of such antibodies (7). This led to collaborative studies in New Haven which established firmly in 1968 that EBV was the etiologic agent of IM (8). The viral tropism for lymphocytes, specifically B cells, was later shown to stem from a receptor closely related to the C3d component of complement for EBV on B lymphocytes (9).

As with man, EBV can infect and transform B lymphocytes "*in vitro*" from a New World non-human primate, the cotton-topped marmoset (10, 11). Cell-free EBV, autologous transformed cells, or cell-associated virus given to these marmosets can induce fatal B cell lymphoproliferative states (12).

After the demonstration in 1970 that patients with nasopharyngeal carcinoma, a highly malignant neoplasm prevalent in certain parts of Asia, had elevated serologic titers of antibodies to EBV (13), it came as no surprise that EBV DNA was identified in the malignant epithelium of these neoplasms (14).

About this time, studies originating in the United States revealed that individuals with compromised immune systems, whether genetically determined (15) or iatrogenically induced (16), were subject to B cell lymphoproliferative malignancies. Only later would EBV be shown to be operative in these states (17–20) and in a newly defined lethal epidemic, AIDS (21).

THE DISCOVERY OF XLP

The definitive work, published in 1975 (22), stemmed from observations drawn over a 6-y period. The index case was defined in 1969 after an autopsy at Children's Hospital of Boston. The autopsy was performed under the supervision of one of the very best pediatric pathologists, the late Gordon F. Vawter, M.D. A previously healthy 8-y-old boy died 30 d after the onset of IM with fulminant hepatitis and marrow failure. The liver, spleen, lymph nodes, and marrow were diffusely infiltrated with immunoblasts, plasma cells, and histiocytes. At the time, Dr. Vawter, despite his vast experience, was unable to cogently ascribe the clinical/autopsy findings to a lengthy list of uncommon pediatric conditions. Not long thereafter, it was learned that two brothers died in early life from an acute illness characterized by features which resembled IM and acute lymphoblastic leukemia. Subsequently, it was learned that three maternally related nephews developed agammaglobulinemia after IM, cerebral lymphoma after IM, and extranodal (ileocecal) lymphoma unrelated to IM. These boys, related to a common ancestor, surname Duncan, defined a new entity (Duncan's disease) characterized by a presumed X chromosomal mutation which places affected males in jeopardy when confronted by EBV. At the time, this clearly was disquieting news, as yet another condition was defined in which this ever-present virus might inflict human disease. The issue then centered upon defining more clearly the disease state in these affected boys, formulating treatment for the vulnerable and, above all, initiating research into its pathogenesis.

THE CREATION OF THE XLP REGISTRY

To track and characterize this disease, a Registry was established in 1980 (23). As computerization advanced, the literature was scoured to identify boys who had inherited what appeared to represent an inability to confront EBV. In due course, this led to a pursuit which covered vast corners of North America, Europe, the Middle East, South America, and Australia.

THE NORMAL IMMUNE RESPONSE TO EBV

Before discussing the clinical phenotypes of XLP, it shall be necessary to describe the normal immune response which contains this ubiquitous virus.

EBV replicates in the oropharyngeal cells (24), from which it is shed into the saliva (25). Infection generally follows exposure to infected saliva, although blood transfusions may also result in EBV infection (26). Given its tropism for B cells, the lymphoid tissue of Waldeyer's ring becomes infected, and the EBV-laden B cells disseminate widely. In most individuals, the immune system responds with silent efficiency, such that no overt clinical state is induced. Most of us produce lifelong IgG antibodies to EBV proteins (VCA and EBNA), a reflection of a steady-state between productive viral cycles and immune recognition, as well as the latent property of this Herpesvirus.

Through studies of patients with IM, the complexity and elegance of this immune response has been defined. At the moment of clinical malaise, lymphadenopathy, pharyngitis, splenic enlargement, and hepatic dysfunction, a consortium of immune elements is well engaged. Early on, natural killer cell and $CD4^+$ T cell activity are heightened (27). At this time, cytokine production is underway and B cells undergo a polyclonal expansion and synthesize IgM and IgG antibodies to EBV-determined proteins. Later on, cytotoxic $CD8^+$ T cells restricted to class I MHC antigens and specific for EBV undergo proliferative expansion to check the B cell infection (27). In due course, the immune response dominates and a "ceasefire" is drawn, commensurate with the regression of adenopathy and splenomegaly and return of hepatic homeostasis. As with quiescent seroconversion, low grade productive and lytic viral cycles are met with immune surveillance, as reflected by the sustained production of IgG antibodies to VCA and EBNA.

CLINICAL PHENOTYPES OF XLP

At this writing, the collected data of the XLP Registry have been totally reassessed and tabulated. Among some 80 families, 272 boys have been defined to have XLP and over 2,500 family members have been studied. The low numbers of affected boys suggest that the disease is rare. Although this is true, we suspect the condition is underdiagnosed and that the true incidence is greater than the Registry data would suggest.

These data allow for a precise description of the diverse phenotypes of XLP (28–30). The most recent tabulation of the Registry is presented in Table 1.

Fulminant IM with VAHS. For XLP boys, their parents, and clinicians the most dramatic expression of XLP takes this form. In contrast to the well orchestrated normal immune response to EBV, these boys mount a dysregulated, exuberant response to the virus, which unleashes $CD8^+$ T cells, EBV-infected B cells, and macrophages in tissues throughout the body. Through the effects of cytotoxic T cells and other associated cytokines, extensive parenchymal damage ensues, most vividly manifest in the liver (as fulminant hepatitis) and bone marrow (as profound hypoplasia). Other tissues are also affected, as infiltrates and cell injury are manifest in the spleen (extensive necrosis of white pulp), brain (perivascular mononuclear cell infiltrates), heart (mild mononuclear cell myocarditis), kidneys (mild interstitial nephritis), and thymus. The latter features thymocyte depletion and necrosis of thymic epithelium (31), not unlike that seen in human (32) and experimental (33) graft-*versus*-host disease and in AIDS (34). The VAHS component features widely disseminated histiocytes replete with erythrocytes and nuclear debris. This element is present in 90% of boys fulminant IM. Nearly half of the XLP boys feature this phenotype; most die within 1 mo post-EBV infection. Until recently, no treatment was available.

Dysgammaglobulinemia. This expression of XLP most often, but not always, follows EBV infection. Affected patients have varying degrees of hypogammaglobulinemia primarily affecting IgG antibodies. Changes in IgM and IgA levels are also present, most commonly manifesting as hyper-IgM. The lymphoid tissues (lymph nodes, white pulp of spleen, thymus, bone marrow) are the site of extensive damage with resulting necrosis and lymphoid depletion. Lymph nodes may also feature extensive areas of calcification. Boys who survive this complication are treated (as those hypogammaglobulinemic pre-EBV infection) with monthly i.v. gammaglobulins.

Malignant lymphoma. This expression of XLP presents a new twist in the spectrum of genetically determined diseases associated with EBV infection. The lymphomas have been uniformly extranodal, most often affecting the ileocecal region (29). Lesser numbers of cases have been tabulated at central nervous system, hepatic, and renal sites (29). These lymphomas are usually of Burkitt's type, although four cases of Hodgkin's disease have occurred. Although most are of B cell origin, some have a T cell phenotype. In at least two cases, the characteristic 8;14 translocation in a Burkitt's lymphoma was found (35, 36). Clinicopathologic features of these lymphomas (extranodal predilection, high grade histology, clonality, gene rearrangement) are similar to those seen in other primary immunodeficiencies. In 18 patients, lymphoma developed without serologic evidence of previous EBV infection. Two of these cases went on to succumb to fatal IM years after cure of their lymphoma.

Aplastic anemia. Distinct from the marrow depletion attending fulminant IM with VAHS, a limited number of boys have developed isolated marrow aplasia (either pancytopenia or pure red cell aplasia) after EBV infection. Regrettably, little is known regarding the pathogenesis of this aplastic state, although it has been shown that nearly one-third of non-XLP patients with sporadic aplastic anemia have EBV in their marrow cells (37).

Vasculitis and pulmonary lymphomatoid granulomatosis. Several boys have developed lymphoid vasculitis resulting in destruction of arterial walls with ensuing aneurysmal dilatation (29). Five have presented with T cell pulmonary lymphomatoid granulomatosis (29). Four were EBV seronegative, and the EBV genome could not be detected in the lesions. One of the patients also manifested lymphomatoid granulomatosis of the central nervous system with extensive damage. Curiously, these lymphoproliferations represented primarily activation of $CD4^+$ T cells and could occur in the absence of EBV exposure.

Sequential phenotypes. Not uncommonly, XLP boys manifest several phenotypes of the disease over time. This phenotypic variation most often includes dysgammaglobulinemia, malignant lymphoma and marrow aplasia. In contrast, most of those who develop IM and VAHS die within 1 mo of onset of symptoms; thus, this phenotype appears to be unique.

PATIENT SURVIVAL

Follow-up data are available for 87% of the boys. Seventy-five percent of the boys have died, some 70% before 10 y of age; only two have lived to 40 y of age. Of the 157 who have developed fulminant IM and VAHS, the most virulent phenotype of XLP, five have recently been successfully treated with chemotherapy, and four have received etoposide (VP-16), with or without immunosuppressive therapy, resulting in quiescent disease and survival. Survival rates for fulminant IM/VAHS, lymphoproliferative disorders, dysgammaglobulinemia, and aplastic anemia are 4, 35, 55, and 50%, respectively (Table 1).

IMMUNODEFICIENCY PRIOR TO EBV EXPOSURE

It was long suspected that XLP boys were immunodeficient even before EBV infection. Several boys developed measles

Table 1. *Phenotypes of 272 XLP boys from 80 families as compiled from the XLP Registry*

Phenotype	Number affected	Mean age at onset (y)	Survival rate
Fulminant IM	157 (58)*	5	5/132 (4)*
Lymphoproliferative disorders (includes five cases of T cell lymphoproliferation and three cases of Hodgkin's disease)	82 (30)	6	25/71 (35)
Dysgammaglobulinemia	84 (31)	9	41/74 (55)
Aplastic anemia	8 (3)	8	4/8 (50)
Vasculitis, lymphomatoid granulomatosis	7 (3)	6.5	2/7 (29)

* Numbers in parentheses are percentages.

pneumonitis, two incurred *Neisseria meningitidis* infection, and one succumbed to disseminated vaccinia after smallpox vaccination (29). From these anecdotal experiences, especially the latter, it was reasoned that these boys harbor an abnormal immune system before an encounter with EBV.

During the 1980s, extensive immunologic studies were performed in these boys, yet few patterns of immune dysfunction emerged. The most consistent defect related to an inability to switch Ig isotype (IgM → IgG) after consecutive exposures to phage ϕX174 (38). Although not specific to XLP, for years this test represented the "gold standard" to identify boys who inherited the mutated gene. As well, the inability to mount an IgG response to EBNA after EBV infection appeared to represent a characteristic trait of the XLP boys, although these two defects may also be seen in other primary immunodeficiencies.

The DNA and serum obtained from the four corners of the world led to interesting data. Some 32 boys, devoid of serologic and, in some cases, genomic evidence for EBV infection, have been found through RFLP analysis to have inherited the mutated gene. Among these, 27 manifest an XLP phenotype: 17 dysgammaglobulinemia (either panhypogammaglobulinemia or hypo-IgG_1 and IgG_3 and hyper-IgM with or without hyper-IgA) and 18 lymphoproliferative disease, including three of T cell origin (39). Thus, even in the absence of EBV infection, these boys feature an inability to regulate Ig gene expression and/or to contain B or T cell lymphoproliferation.

THE DIAGNOSIS OF XLP

Upon assuming charge of the Registry, we deemed it important to define the criteria for establishing the diagnosis of XLP. Objective elements involved family history, clinical findings, genotypic analyses, and serologic studies to quantitate Igs, EBNA antibodies after EBV infection, and assess for Ig isotype switch after exposure to phage ϕX174. These criteria, akin to those established decades ago for the diagnosis of acute rheumatic fever, were published in 1993 (30) and are presented in Table 2.

DETECTION OF THE CARRIER STATE IN FEMALES

As time passed, a critical and not insignificant issue centered about determining the carrier state of young women, usually sisters of XLP boys. Fortunately, this need coincided with progress in the genotypic detection of the disease state. Thus, since the early 1990s, females at risk have been genotyped using DNA probes and RFLP analyses to loci [DXS42, DXS37, and DXS12 (40) and, more recently, DXS10 and DXS100] near the XLP gene. In families in which the clinical criteria can be met and RFLP analysis is informative, a strong presumptive determination can be made regarding carrier status.

THERAPY FOR XLP

Until recently, treatment of XLP boys has been painfully disappointing. Antiviral agents such as acyclovir, IFN-γ and -α, and Ig administered at the onset of EBV infection have been ineffective. Empiric therapy with parenteral Ig rich in anti-EBV antibodies has been given to EBV-negative and -positive boys based on evidence that maternal antibodies protect the newborn infant from primary EBV infection for 4–6 mo. Even this is not absolute, because several boys have succumbed to fulminant EBV infection and others have become infected with EBV while under this putative Ig prophylaxis. Therefore, it is our current recommendation that Ig therapy is not necessary, except in the cases of dysgammaglobulinemia.

Initially, results in the treatment of malignant lymphoma of XLP were extremely poor. However, with current standard chemotherapy protocols for the treatment of pediatric lymphomas, remission is often achieved. These remissions can be durable, but relapse or development of other manifestations of XLP are nearly universal.

The use of etoposide (to quell macrophage activation) and/or T cell immunosuppression (cyclosporin A) have been employed successfully in treating boys with severe acute IM/VAHS or aplastic exacerbations of XLP; however, there is at least one case in which this approach was unsuccessful. Notwithstanding this setback, the use of etoposide is recommended, as one has but to read of the dramatic salvage of a young boy with severe acute IM and VAHS who responded to the drug. The acute symptoms were quenched, and, in due course, he underwent successful bone marrow transplantation (41).

Currently, the only curative therapy is allogeneic hematopoietic stem cell transplantation (36, 41, 42). Some eight affected male patients have undergone stem cell transplantation (six sibling marrow donors, one unrelated marrow donor, one

Table 2. *Criteria for establishing a diagnosis of XLP*

Diagnosis of XLP	Criteria
Definitive diagnosis of XLP	Two or more maternally-related males manifest an XLP phenotype following EBV infection
Probable diagnosis of XLP:*	
Major criteria	Strong genetic linkage to the XLP locus in male with informative markers; XLP phenotype in male after EBV infection
Minor criteria	Hyperimmunoglobulinemia A or M (before EBV infection); hypoimmunoglobulinema G1 or G3 (before EBV infection); inadequate anti-Epstein-Barr nuclear antigen Ig response after EBV infection; failure to undergo IgM → IgG switch after secondary challenge with ϕX174
Possible diagnosis of XLP	Any male maternally related to a male manifesting XLP phenotype

* Diagnosis requires both major criteria or one major and two minor criteria.

umbilical cord blood from sibling). Age at time of transplant appears to be critical; all four boys less than age 15 are alive and well 2 y or more post-transplant, whereas all four greater than 15 y of age died within 90 d of complications of transplantation (Gross TG, unpublished observations).

THE X CHROMOSOME AND IMMUNODEFICIENCY

It is now apparent that the X chromosome is a major repository of genes vital to immune function. This reasoning stems from the fact that most immunodeficiencies are X-linked and/or autosomal recessive. Thus, males are clearly at a heightened risk to inherit mutated genes central to thwarting infectious agents. This led to the concept that there exists an immunologic basis for the superior survival of females (43).

As things stand, some six conditions stemming from mutant genes have been defined. Five of these genes have been cloned; four relate to lymphocytes and one involves neutrophils. In all instances, the functional properties of the affected cells are subverted and severe disease, often fatal, is the outcome.

The first condition, chronic granulomatous disease, may present in both an X-linked and an autosomal recessive form. As for the former, the responsible gene, which encodes for cytochrome B in neutrophils, normally enables these scavengers to release superoxides in response to bacterial infections. This gene was found to be mutant in those boys who incur recurrent bacterial and fungal infections (44).

In 1993 and 1994, four additional X-linked genes relating to immunodeficiency were cloned.

In X-linked agammaglobulinemia, the mutated gene, ATK (adenosine tyrosine kinase), a member of the *src* family of oncogenes, prevents affected boys from expressing a particular intracellular tyrosine kinase in their B cells which is developmentally regulated and required for B cell differentiation (45). B cell maturation is therefore halted at the pre-B cell stage, thereby leading to a block in the synthesis of all Ig, resulting in recurrent episodes of pyogenic infections.

In the condition known as hypergammaglobulinemia M, Ig isotype switching is blocked, resulting in the production, generally excessive, of IgM and IgD, yet very low or absent levels of IgG, IgA, and IgE. The process of isotype switching involves several signals, one of which is the binding of a tumor necrosis factor-related activator protein (TRAP) on T cells to the CD40 ligand on B cells. Mutation of the TRAP gene constitutes the molecular basis for the failure in isotype switching (46), manifesting clinically by recurrent bacterial infections and pathologically by the absence of germinal centers in lymphoid tissues.

In the X-linked form (autosomal recessive forms also exist) of severe combined immunodeficiency, the quintessential immune deficiency, mutation of the gene encoding the γ chain of IL-2 receptor constitutes the molecular basis of the inability to generate normally functioning mature T cells (47). Since this γ chain is shared by the IL-7 and IL-4 receptors, the defect results in an inability to respond to IL-2, IL-4, and IL-7. In these patients, the B cells produced are akin to neonatal B cells and Ig production may be impaired. Failure to thrive, diarrhea, and recurrent, ultimately fatal, viral/fungal/parasitic infections stem from this mutation.

In Wiskott-Aldrich syndrome, boys suffer from severe eczema, thrombocytopenia, and recurrent pyogenic, viral, and protozoan infections. Circulating leukocytes have a defective expression of sialophorin, a CD43 transmembrane mucin-like molecule (48) necessary for cell-cell contacts and T cell and monocyte activation. Because the gene for sialophorin resides on 16p, this CD43 abnormality cannot represent the sole molecular defect in this condition. Indeed, the gene for Wiskott-Aldrich syndrome, which was mapped to Xp11.22–11.3, has been recently cloned and found to encode for a 501-amino acid protein pivotal to lymphocyte and platelet function (49).

The final X-linked immunodeficiency condition is XLP. As discussed subsequently, neither the molecular basis for the disease nor the identity of the gene is as yet known.

The locations of the genes responsible for these X-linked conditions are illustrated in Figure 1.

THE SEARCH FOR THE GENE IN XLP

The inheritance pattern of XLP allowed an instant assignment of the gene to the X chromosome, narrowing its position from anywhere in 3000 Mb, which is the size of the human genome, to 150 Mb, the size of the human X chromosome.

Skare *et al.* (50) provisionally mapped the gene responsible for XLP to the long arm of the X chromosome between Xq24 and Xq26. In 1989, the same group narrowed down the critical region of the XLP gene to approximately 15 Mb around DXS42 and DXS37, using restriction fragment length polymorphisms (51, 52). Although most of the affected individuals show no structural defect of the X chromosome, Wyandt *et al.* found one family (Registry #43) with partial deletion of Xq25–26 accompanying XLP (53, 54).

Subsequently, much progress has been made in the genetic mapping of the XLP gene in Xq25. Prior study of seven kindreds linked the XLP gene to DXS42 (LOD 17.5) and DXS37 (LOD 13.3) (52). In the genetic map, DXS37 is proximal (centromeric) to DXS42. Physical mapping indicated that DNA markers DXS42 and DXS37 were present in the index XLP family with the interstitial deletion. However, the marker loci for DXS6, DXS739, and DXS100 were absent in the male patient (Registry #43–004) with the Xq25 deletion. Three overlapping deletions found in Skare's laboratory soon thereafter established the gene locus and refined its location to a 2.5-Mb region in Xq25 (55).

To be able to construct a physical map of the XLP critical region, several YAC clones have been isolated carrying DNA sequences deleted in the X chromosome of Registry patient #43–004. DNA probes from the region specific YAC in combination with DNA markers with known locations were used to establish a physical map of the region using interphase fluorescence *in situ* hybridization (56). The authors suggested the following order in the region: *cen*-(DXS12, DXS42)-DXS6-DXS982-DXS739-DXS75-DXS100-DXS10-DXS177-*tel*. Using DNA probes from the region, additional XLP patients with interstitial deletions were identified by Skare *et al.* (55) and in

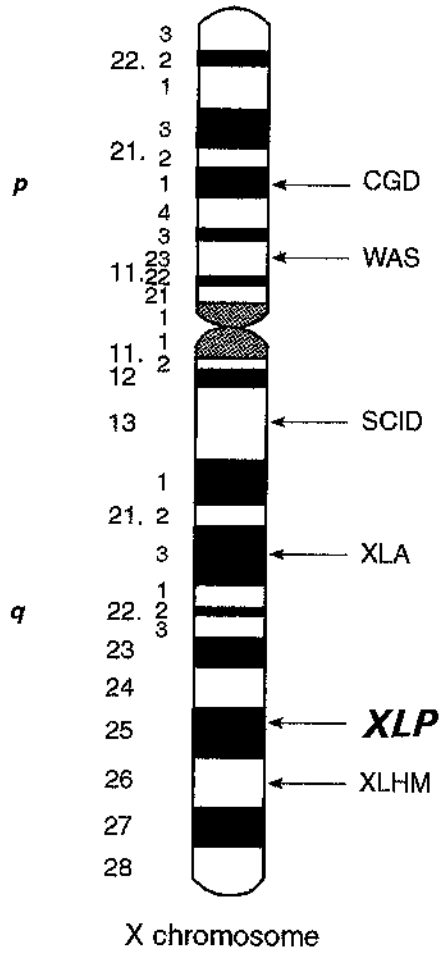

Figure 1. Schematic representation of the X chromosome, depicting six loci central to immune function. Each of these genes, except for XLP (indicated in *italics*), has been cloned. Abbreviations: CGD = chronic granulomatous disease, SCID = severe combined immunodeficiency, WAS = Wiskott-Aldrich syndrome, XLA = X-linked agammaglobulinemia, XLHM = X-linked hypergammaglobulinemia M, XLP = X-linked lymphoproliferative disease. This schematic is published with permission of Mosby-Year Book Publishing Company, Philadelphia, PA, as it shall appear in Chapter 27, "Primary Immunodeficiencies," of the forthcoming 10th edition of *Anderson's Textbook of Pathology* (I. Damjanov and J. Linder, eds). The figure was designed by the medical illustrator, Ms. Mary Jean McFadden, and is copyright 1995, Mosby-Year Book Publishing Company.

our own laboratory (Sumegi J, unpublished). It was concluded that the XLP candidate gene region spans the region between DXS6 and DXS100 and corresponds to approximately 2 Mb of DNA. This region has been completely covered by overlapping YAC clones (Sumegi J, unpublished); hence, it is but a matter of time before the gene is cloned.

HYPOTHESIS

Recently, it has been recognized that the immune response to infection is regulated by a balance between the TH1 and TH2 cell responses. The phenotypes of the specific cells responsible for these divergent responses have not been discerned. However, TH1 responses are characterized by the secretion of cytokines (*e.g.* IL-2 and IFN-γ) and are responsible for cell-mediated immune responses. TH2 responses are characterized by the secretion of cytokines (*e.g.* IL-4 and IL-10) required in an humoral immune response (57, 58). In addition, these two types of responses counterbalance each other (*i.e.* TH2 down-regulates TH1 and vice versa).

The predominant type of response (TH1 *versus* TH2) has been shown to have clinical significance in various types of infections. Infections with intracellular organisms are less severe when the TH1 response predominates. For example, people infected with *Mycobacterium leprae* can display a spectrum of clinical manifestations. In tuberculoid leprosy, which features local lesions, the TH1 response predominates, but in disseminated disease, lepromatous leprosy, the TH2 response is predominant (59, 60). Similar results have been shown in infections with *Mycobacterium tuberculosis* (60–62) and leishmania (58, 60, 63). However, in extracellular infections (*e.g.* helminth parasitic infections), the TH2 response is associated with less severe disease (60, 64).

We suspect, because EBV is an intracellular infection, that the TH1 response is important in the initial control of the infection. IFN-γ has been shown to be elevated in the serum of a patient following primary infection; however, it was only detectable during the incubation period and not when symptoms of acute IM developed (65). This suggests that the TH1 response may be important in controlling the early infection, but is normally downregulated, probably by the TH2 response, resulting in persistent humoral immunity, as is normally observed. In contrast, in three out of five XLP patients with fatal IM, elevated levels of IFN-γ were detected (66). Characteristically, XLP patients who survive primary infection do not mount an appropriate humoral response to EBV (*i.e.* low titers and/or absent antibodies to EBNA) (19, 29, 67).

It is our hypothesis that the defect in XLP resides in the inability to effect an appropriate TH2 response after infection with EBV. As a result, the TH1 response is uncontrolled, devoid of regulation, and either leads to parenchymal dysfunction (fatal IM) or results in dysgammaglobulinemia, aplastic anemia and dysregulation of other immune functions (natural killer and cytotoxic T cell activity) predisposing to lymphoma.

CONCLUDING REMARKS

The XLP story portrays an interesting chapter in medical science. The disease, initially discovered at the autopsy table, is now the subject of hot pursuit in the molecular laboratory. The XLP Registry data reveal that the mutated gene renders boys vulnerable to dysimmunoregulation and lymphoproliferative lesions, some before and all after EBV infection. Given the near universal infection of man by the virus, there are compelling reasons to pursue the quest of this gene, to establish its structure and, above all, to determine its function.

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considerable strengths toward the elucidation of its pathogenesis and treatment over the next 22 years. The DNA he obtained from affected boys shall make possible the cloning of the gene, either by us or others with whom he generously shared material. On his death in 1992, he had assembled a group to carry forth his mission. In may ways, this is his story, if not his legacy.

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