

BRIEF REPORT: CORRECTION OF X-LINKED HYPER-IgM SYNDROME BY ALLOGENEIC BONE MARROW TRANSPLANTATION

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THE X-linked hyper-IgM syndrome is a rare immunodeficiency disease in which the ability of B cells to switch immunoglobulin production from IgM to IgG, IgA, and IgE is defective.¹ A variety of mutations of the gene encoding the CD40 ligand cause the immunodeficiency.²⁻⁶ The functional effect of the mutation is that the CD40 ligand on T cells cannot interact with the CD40 glycoprotein on the surface of B cells. This interaction normally mediates immunoglobulin class switching by B cells. The deficiency of IgG and IgA leads to recurrent infections of the respiratory tract that can be prevented by intravenous immune globulin.¹ Patients with the X-linked hyper-IgM syndrome are also prone to neutropenia, autoimmune disorders, and lymphomas.^{1,7} Some are susceptible to infection with opportunistic microorganisms such as *Pneumocystis carinii*, *Histoplasma capsulatum*, and cryptosporidium.^{1,8-13} Cryptosporidium causes unremitting diarrhea and is associated with cholangitis and cirrhosis. Although cellular immunity is normal in the X-linked hyper-IgM syndrome, the occurrence of these kinds of infections suggests a T-cell defect, possibly related to impaired interactions between T cells and macrophages¹⁴ and epithelial cells mediated by the CD40 glycoprotein and the CD40 ligand.¹⁵

Allogeneic bone marrow transplantation has the potential to cure genetic disorders affecting marrow-derived cells, such as β -thalassemia,¹⁶ severe combined immunodeficiency,¹⁷ the Wiskott-Aldrich syndrome,¹⁸ the X-linked lymphoproliferative syndrome,¹⁹ and leukocyte-adhesion deficiency.²⁰ We performed allogeneic bone marrow transplantation in a child with the X-linked hyper-IgM syndrome who was at risk for life-threatening opportunistic infections.

CASE REPORT

A five-month-old boy (Subject III-3 in Fig. 1) was given a diagnosis of X-linked hyper-IgM syndrome on the basis of low serum concentrations of IgG and IgA (Table 1) and the family history. Two maternal uncles (Subjects II-6 and II-7 in Fig. 1) had died of protracted diarrhea at the respective ages of six months and two years; both had hypogammaglobulinemia. A first cousin (Subject III-1) was also

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given a diagnosis of X-linked hyper-IgM syndrome on the basis of low serum IgG and IgA concentrations. He has persistent diarrhea caused by cryptosporidium and cholangitis associated with liver cirrhosis. He receives parenteral nutrition and is under consideration for liver transplantation.

The diagnosis of X-linked hyper-IgM syndrome was confirmed by the finding that the expression of the CD40 ligand on the patient's activated T lymphocytes was defective (Fig. 2). A mutation in the gene that encodes the CD40 ligand was found in Subject III-1³; the CD40 ligand transcripts in that patient's T cells had a deletion of 10 base pairs (bp) in the extracellular domain of the CD40 ligand (nucleotides 447 to 456). The intragenic CD40 ligand microsatellite probe revealed a polymorphism in the family. A 164-bp allele (allele 2) was identified in affected Subjects III-1 and III-3 (Fig. 3).²¹

Subject III-3 was treated with intravenous immune globulin every three weeks beginning at the age of five months. At the age of seven months *P. carinii* pneumonitis developed; the infection was cured by treatment with trimethoprim-sulfamethoxazole (Bactrim). His growth and general status remained satisfactory. The HLA haplotype of his healthy sister, born in October 1992, was HLA-A, B, DR, DQ, DP — identical to that of the patient. Her blood group was A-positive; the patient's blood group was O-negative.

Because of the family history of two fatal cases of X-linked hyper-IgM syndrome and the occurrence of an opportunistic infection in the patient, the parents gave informed consent for the patient to undergo bone marrow transplantation, with his sister as the donor. The conditioning regimen consisted of busulfan (5 mg per kilogram of body weight per day for four days beginning nine days before transplantation) and cyclophosphamide (Endoxan) (50 mg per kilogram per day for four days beginning five days before transplantation). Prophylaxis against graft-versus-host disease consisted of cyclosporine (initial dose, 3 mg per kilogram per day as a continuous infusion, followed by a dose of 6 mg per kilogram per day orally beginning 1 day before transplantation and continuing for 180 days afterward) and methotrexate (10 mg per square meter of body-surface area on days 1, 3, 6, and 11 after transplantation). Because of the incompatibility between red-cell groups (the donor was A-positive and the recipient O-negative), the donor's marrow inoculum was depleted of erythrocytes on Plasmagel (gelatin in glucose). On December 8, 1993, the patient received 3.2×10^8 nucleated marrow cells per kilogram from his sister, who was negative for cytomegalovirus and Epstein-Barr virus. Prophylaxis against infection included hospitalization in a Trexler's isolation unit, oral administration of nonabsorbable antibiotics, and weekly treatment with intravenous immune globulin (200 mg per kilogram) for four months.

METHODS

Polymorphonuclear cells and mononuclear cells were isolated from heparin-treated blood by exposure to dextran followed by Ficoll-Hypaque centrifugation. An E-rosette assay was performed by incu-

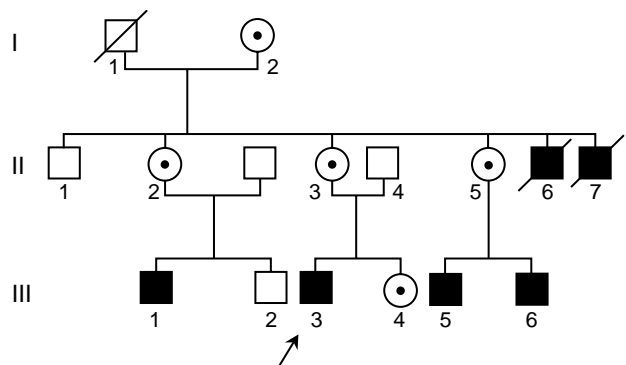


Figure 1. Pedigree of a Family with the X-Linked Hyper-IgM Syndrome.

Squares denote male family members, circles female members, solid squares members with the X-linked hyper-IgM syndrome, circles with a dot female carriers, and the arrow the proband.

Table 1. Serum Immunoglobulin Concentrations and in Vivo Antibody Responses in the Patient before and after Bone Marrow Transplantation.*

VARIABLE	DATE								VALUES FOR AGE-MATCHED CONTROLS†
	1/91	11/93	1/94	3/94	5/94	9/94	12/94	2/95	
IgG (mg/dl)	0.53	11.7	11.8	10.3	5.7	10	11.5	6.20	9.29±2.28
IgA (mg/dl)	0.07	0.08	0.11	<0.08	<0.08	0.32	0.54	0.35	0.56±0.18
IgM (mg/dl)	0.58	1.39	0.55	0.57	0.62	2.17	1.85	0.60	0.71±0.37
IgE (IU/ml)‡		<2				3		3	
Isohemagglutinin titer (×10 ⁻¹)	1§						4§		4–16
Tetanus toxoid (IU/ml)	ND					0.25			>0.1
Poliovirus-vaccine titer (×10 ⁻¹)	ND								
Type 1							20		>40
Type 2							80		>40
Type 3							10		>40

*Intravenous immune globulin (200 mg per kilogram per week) was given from December 8, 1993, to March 21, 1994. Bone marrow transplantation was performed on December 8, 1993. ND denotes not done.

†Plus-minus values are means ±SD.

‡To convert values for serum IgE to micrograms per liter, multiply by 2.4.

§Anti-B isohemagglutinins.

bating mononuclear cells with neuraminidase-treated red cells from sheep.

Monocytes and B lymphocytes were isolated by sorting with a CD14-specific LeuM3 antibody (Becton Dickinson, San Diego, Calif.) and a CD19-specific antibody (Becton Dickinson), respectively, in a FACStar Plus cell sorter (Becton Dickinson).

The expression of the CD40 ligand was evaluated with a CD40-immunoglobulin fusion protein (CD40-Fc)²² as described previously,³ after a five-hour incubation of E-rosette-forming cells with phorbol myristate acetate and ionomycin. A polymorphism of the CD40 ligand microsatellite CA repeat²¹ was studied after amplification with the polymerase chain reaction with two primers flanking the CA repeat located in the 3' untranslated region of the CD40 ligand gene. Products were analyzed on 5 percent denaturing polyacrylamide gels.

Fluorescence in situ hybridization was performed with an X-chromosome α -satellite probe (DX21) and a Y-chromosome-cocktail probe (DY23 and DY21) labeled with biotin and digoxigenin, respectively (Oncor, Gaithersburg, Md.). Hybridization was carried out according to the manufacturer's recommendations. After overnight hybridization and post-hybridization washes, the slides were incubated in blocking solution (0.1 percent phosphate-buffered saline, 20.5 percent Tween, and nonfat dry milk). The X-chromosome probe was detected with avidin-Texas red (Vector Laboratories, Burlingame, Calif.), and the Y-chromosome probe with mouse antidigoxigenin fluorescein-labeled antibody (Boehringer-Mannheim, Mannheim, Germany). The slides were mounted with an antifade solution containing 1 μ g of 4',6-diamidino-2-phenylindole per milliliter, examined with a Leitz microscope (model DM, Leitz, Rockleigh, N.J.), and analyzed with a Cytovision computer (Imaging International, Sunderland, United Kingdom).

RESULTS

The patient's clinical course after bone marrow transplantation was uneventful. The absolute granulocyte count exceeded 500 per cubic millimeter by day 26, and the last platelet transfusion was given on day 20. Manifestations of neither acute nor chronic graft-versus-host disease occurred. No infectious complications were seen. The patient was sent home on day 35. Seventeen months after bone marrow transplantation, he is doing well, with normal blood counts and no need for therapy.

Bone marrow engraftment was demonstrated by several means. The patient's red-cell group changed from

O-negative to A-positive. In this boy, fluorescence in situ hybridization with probes specific for the X and Y chromosomes showed that on day 360, 100 polymorphonuclear neutrophils and 100 peripheral-blood mononuclear cells were all positive for the X-chromosome probe and negative for the Y-chromosome probe. The same result was found on 50 separated E-rosette-forming cells and 50 cells that did not form E rosettes (data not shown).

Twelve months after bone marrow transplantation, examination of the CA-repeat polymorphism associated with the CD40 ligand showed a shift from allele 2 (associated with a mutation of the CD40 ligand gene) before bone marrow transplantation to alleles 2 and 3, which were present in the carrier donor (Fig. 3). The same change was detected in E-rosette-forming cells, cells that did not form E rosettes, and granulocytes. Expression of the CD40 ligand by activated T cells from the recipient one year after bone marrow transplantation was equivalent to the expression of the ligand by the donor's T cells (Fig. 2). In both children, expression of the ligand was lower than in control subjects, because of the donor's carrier status (Fig. 2).

The patient had a full recovery of immune function six months after bone marrow transplantation. By then he had normal T-cell and B-cell counts; antigen-induced T-cell proliferation in vitro; normal serum concentrations of IgG, IgA, and IgE after the cessation of intravenous immune globulin therapy; and normal antibody responses to immunization with poliovirus and tetanus toxoid (Table 1).

DISCUSSION

We report successful allogeneic bone marrow transplantation in a child with the X-linked hyper-IgM syndrome. To reduce the risks of long-term sequelae, the conditioning regimen consisted only of chemotherapy, in accordance with a regimen used in other patients with various immunodeficiencies.²³ Full engraftment

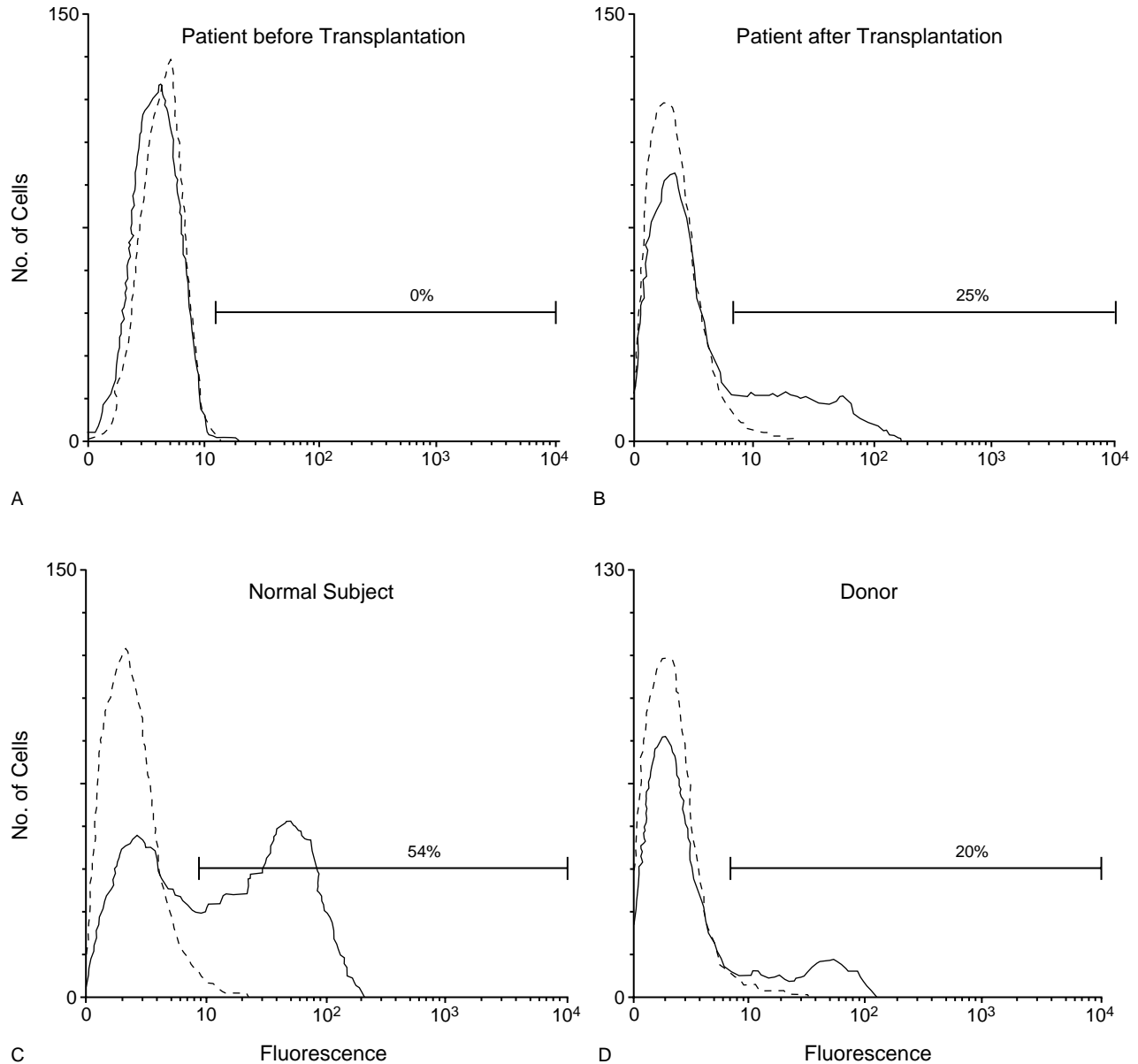


Figure 2. Expression of the CD40 Ligand by Activated T Cells from the Patient before and after Bone Marrow Transplantation, the Donor, and an Age-Matched Control Subject.

Binding of CD40-immunoglobulin fusion molecules to activated T cells was measured in the patient before (Panel A) and one year after (Panel B) bone marrow transplantation, in a normal subject (Panel C), and in the donor (Panel D). Binding was measured by fluorescence in situ hybridization on electronically gated CD3⁺ cells. Fluorescence was measured in arbitrary units. Dashed lines denote unstained cells, and solid lines activated T cells. The values above the bars are the percentages of positive cells.

was shown by several means, including changes in red-cell antigens, the results of fluorescence in situ hybridization for X and Y chromosomes, polymorphism of the CD40 ligand gene, and expression of the CD40 ligand by activated T cells. After transplantation, expression of the ligand by the recipient's T cells was equivalent to that by the donor's T cells, which had reduced expression because of her carrier status. In female carriers of the mutant CD40 ligand gene, a variable fraction of T cells expresses the CD40 ligand because of random inactivation of the X chromosome.²⁴ As expected on the basis of the normal immunologic and clinical status of carriers of the X-linked hyper-IgM syndrome,

the reduced number of T cells expressing the CD40 ligand in the donor was nevertheless sufficient to provide signals for switching to the production of IgG, IgA, and IgE²⁵ and normalizing immune functions.

Not all patients with X-linked hyper-IgM syndrome have life-threatening opportunistic infections like the family of the proband.¹ It has not been possible to correlate a severe clinical outcome with given genotypes.^{3,6,25} The frequency of opportunistic infections in X-linked hyper-IgM syndrome is not known. In a recent summary, *P. carinii* pneumonitis developed in 8 of 67 patients.¹ Infections caused by *H. capsulatum*, aspergillus, cryptococcus, and toxoplasma have also been re-

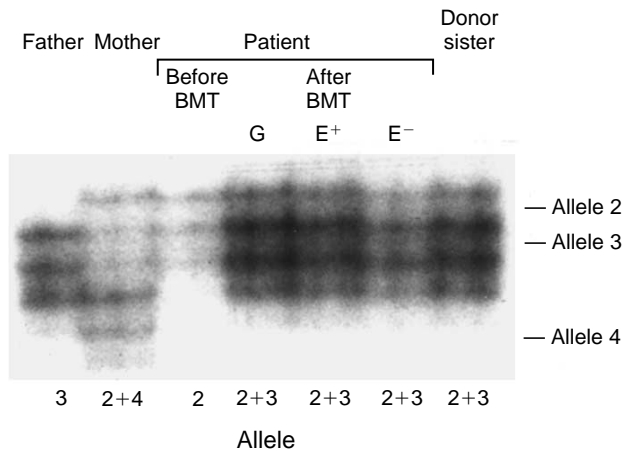


Figure 3. Microsatellite Typing before and after Bone Marrow Transplantation (BMT) of Granulocytes (G), E-Rosette-Forming Cells (E⁺), and Cells That Did Not Form E Rosettes (E⁻) from the Patient, His Parents, and His Sister.

The patient's sister was the bone marrow donor. Allele 2 is linked to a mutation of the intragenic CD40 ligand microsatellite. Before transplantation, the patient had allele 2 and the donor had alleles 2 and 3. After transplantation, the patient had alleles 2 and 3 in granulocytes, E-rosette-forming lymphocytes, and lymphocytes that did not form E rosettes.

ported in this syndrome.^{1,8-13} In a recently reported series, 5 of 16 patients died of either encephalitis or cholangitis with liver failure in a 14-year period.¹³ In our own experience, severe cholangitis associated with cryptosporidial infections developed in 3 of 12 patients (including the first cousin of the child described in this report). One died of liver failure. Liver transplantation in patients with the X-linked hyper-IgM syndrome has been reported.^{13,26}

The relation between the clinical severity of the X-linked hyper-IgM syndrome and the genotype is not obvious, because a large number of different mutations have been described in the disease. Therefore, the formulation of a prognosis in an individual case requires great caution. Because of the poor outcome in the patients with X-linked hyper-IgM syndrome in the family of the propositus and the occurrence of *P. carinii* pneumonitis in the patient, we assumed that he was susceptible to further opportunistic infections. Some unknown genetic factors might modify the clinical consequences of the X-linked hyper-IgM syndrome from a benign course to a severe T-cell immunodeficiency, as we observed in this family.

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REFERENCES

- Notarangelo LD, Duse M, Ugazio AG. Immunodeficiency with hyper-IgM (HIM). *Immunodef Rev* 1992;3:101-21.
- Korthauer U, Graf D, Mage HW, et al. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature* 1993; 361:539-41.
- DiSanto JP, Bonnefoy JY, Gauchat JF, Fischer A, de Saint Basile G. CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. *Nature* 1993;361:541-3.
- Allen RC, Armitage RJ, Conley ME, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993;259:990-3.
- Aruffo AM, Farrington M, Hollenbaugh D, et al. The CD40 ligand gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 1993;72:291-300.
- Fuleihan R, Ramesh N, Loh R, et al. Defective expression of the CD40 ligand in X chromosome-linked immunoglobulin deficiency with normal or elevated IgM. *Proc Natl Acad Sci U S A* 1993;90:2170-3.
- Filipovich AH, Mathur A, Kamat D, Kersey JH, Shapiro RS. Lymphoproliferative disorders and other tumors complicating immunodeficiencies. *Immunodeficiency* 1994;5:91-112.
- Marshall WC, Weston HJ, Bodian M. *Pneumocystis carinii* pneumonia and congenital hypogammaglobulinemia. *Arch Dis Child* 1964;39:18-25.
- Levitt D, Haber P, Rich K, Cooper MD. Hyper IgM immunodeficiency: a primary dysfunction of B lymphocyte isotype switching. *J Clin Invest* 1983; 72:1650-7.
- Benkerrou M, Gougeon ML, Griscelli C, Fischer A. Hypogammaglobulinémie G et A avec hypergammaglobulinémie M. *Arch Fr Pédiatr* 1990;47: 345-9.
- Tu RK, Peters ME, Gourley GR, Hong R. Esophageal histoplasmosis in a child with immunodeficiency with hyper-IgM. *AJR Am J Roentgenol* 1991; 157:381-2.
- Hostoffer RW, Berger M, Clark HT, Schreiber JR. Disseminated Histoplasma capsulatum in a patient with hyper IgM immunodeficiency. *Pediatrics* 1994;94:234-6.
- Banatvala N, Davies J, Kanariou M, Strobel S, Levinsky R, Morgan G. Hypogammaglobulinemia associated with normal or increased IgM (the hyper IgM syndrome): a case series review. *Arch Dis Child* 1994;71:150-2.
- Alderson MR, Armitage RJ, Tough TW, Strockbine I, Fanslow WC, Spriggs MK. CD40 expression by human monocytes: regulation by cytokines and activation of monocytes by the ligand for CD40. *J Exp Med* 1993;178:669-74.
- Schriever F, Freedman AS, Freeman G, et al. Isolated human follicular dendritic cells display a unique antigenic phenotype. *J Exp Med* 1989;169: 2043-58.
- Lucarelli G, Galimberti M, Polchi P, et al. Bone marrow transplantation in patients with thalassemia. *N Engl J Med* 1990;322:417-21.
- Fischer A, Landais P, Friedrich W, et al. European experience of bone-marrow transplantation for severe combined immunodeficiency. *Lancet* 1990; 336:850-4.
- Mullen CA, Anderson KD, Blaese RM. Splenectomy and/or bone marrow transplantation in the management of the Wiskott-Aldrich syndrome: long-term follow-up of 62 cases. *Blood* 1993;82:2961-6.
- Williams LL, Rooney CM, Conley ME, Brenner MK, Krance RA, Heslop HE. Correction of Duncan's syndrome by allogeneic bone marrow transplantation. *Lancet* 1993;342:587-8.
- Le Deist F, Blanche S, Keable H, et al. Successful HLA nonidentical bone marrow transplantation in three patients with the leukocyte adhesion deficiency. *Blood* 1989;74:512-6.
- DiSanto JP, Markiewicz S, Gauchat J-F, Bonnefoy J-Y, Fischer A, de Saint Basile G. Prenatal diagnosis of X-linked hyper-IgM syndrome. *N Engl J Med* 1994;330:969-73.
- Lane P, Traunecker A, Hubele S, Inui S, Lanzavecchia A, Gray D. Activated human T cells express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes. *Eur J Immunol* 1992;22:2573-8.
- Fischer A, Landais P, Friedrich W, et al. Bone marrow transplantation (BMT) in Europe for primary immunodeficiencies other than severe combined immunodeficiency: a report from the European Group for BMT and the European Group for Immunodeficiency. *Blood* 1994;83:1149-54.
- Hendriks RW, Kraakman MEM, Craig IW, Espanol T, Schuurman RKB. Evidence that in X-linked immunodeficiency with hyperimmunoglobulinemia M the intrinsic immunoglobulin heavy chain class switch mechanism is intact. *Eur J Immunol* 1990;20:2603-8.
- Callard RE, Smith SH, Herbert J, et al. CD40 ligand (CD40L) expression and B cell function in agammaglobulinemia with normal or elevated levels of IgM (HIM): comparison of X-linked, autosomal recessive, and non-X-linked forms of the disease, and obligate carriers. *J Immunol* 1994;15: 3295-306.
- Espanol T, Carrera M, Muntane C, Caragol I, Hernandez M, Bertran JM. Opportunistic infections and autoimmune diseases in Hyper IgM syndrome. Presented at the Sixth World Health Organization International Workshop on Primary and Acquired Immunodeficiency Diseases, Orvieto, Italy, June 18-21, 1994. abstract.