Analysis of X-chromosome inactivation in X-linked immunodeficiency with hyper-IgM (HIGM1): evidence for involvement of different hematopoietic cell lineages

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Summary. The pattern of X-chromosome inactivation was analyzed, by means of two different DNA probes (pSPT-PGK and M27β), in several cell lineages derived from females belonging to a pedigree with X-linked immunodeficiency with hyper-IgM (HIGM1). Non-random X-chromosome inactivation was demonstrated in T cells, B cells, and neutrophils, but not in fibroblasts, of obligate carriers, suggesting that different hematopoietic cell lineages are primarily involved in HIGM1. Preferential inactivation of the paternally derived X-chromosome was demonstrated by analysis of segregation of the alleles defined by the pSPT-PGK and M27β probes. The possibility that the HIGM1 mutation may confer a proliferative and/or differential advantage to hematopoietic precursors carrying the mutated allele on the active X-chromosome is discussed.

Introduction

Immunodeficiency with hyperimmunoglobulinemia M is a rare disease characterized by the absence of IgG and IgA, and normal or high levels of serum IgM and IgD (Eibl et al. 1989). In most instances, the disease is transmitted as an X-linked trait, but autosomal recessive, sporadic, or acquired cases have also been reported. Clinically, affected subjects present with recurrent bacterial infections after the first year of life. Neutropenia, oral ulcers, opportunistic infections, autoimmune-like disorders, tonsillar hypertrophy and neoplasms at an early age are also characteristic features that distinguish immunodeficiency with hyper-IgM from other immunoglobulin deficiencies, in particular from X-linked agammaglobulinemia (AGMX1).

The molecular basis for the syndrome is still debated. In particular, whereas an intrinsic defect of the B cells to switch from IgM to IgG-IgA production was originally postulated (Geha et al. 1979; Krantman et al. 1980; Levitt et al. 1983), other studies have suggested that T cells of the patients may be unable to provide the relevant signals to induce the immunoglobulin switch in the B cells (Mayer et al. 1986). Finally, the basis for neutropenia is also unknown; it may result from a stem-cell defect (Stiehm and Fudenberg 1966) or represent an autoimmune phenomenon (Goldman et al. 1967).

Molecular genetic analysis has been attempted in the X-linked variant of immunodeficiency with hyper-IgM (HIGM1, McKusick’s catalog no. 30824). By means of restriction fragment length polymorphism analysis, the gene responsible for HIGM1 has been tentatively assigned to the Xq24–27 region of the X chromosome, with a multipoint lod score of 2.94 at locus DXS42 (Mensinck et al. 1987).

Obligate carriers of HIGM1 are clinically and immunologically indistinguishable from normal females. In obligate carriers of other X-linked immunodeficiencies (namely, X-linked severe combined immunodeficiency, AGMX1, and the Wiskott-Aldrich syndrome), the X-chromosome carrying the mutation is non-randomly inactivated in the cell lines affected by the disease (reviewed by Conley and Puck 1988c). This may explain why, in these diseases, obligate carriers do not show any clinical or laboratory sign of immunodeficiency. In the present study, we investigated the pattern of X-chromosome inactivation in the females of a family with HIGM1, in an attempt to improve the characterization of the pathogenesis of the disease.

Materials and methods

Figure 1 shows the pedigree with HIGM1 investigated in the present study. In addition to the absence of IgA, extremely low IgG serum levels, normal IgM serum levels, and neutropenia (410–670/mm³) were present in the proband III.13. T cell function was normal in both patients III.5 and III.13. Subjects II.6 and II.15 had died because of infections within 18 months of age; subject III.4,
Heparinized or citrated blood was collected from several family members. Neutrophils and mononuclear cells were separated from heparinized blood by dextran sedimentation and Ficoll-Hypaque gradient centrifugation. Mononuclear cells were further separated into T-cell enriched and B-cell enriched populations by the E-rosetting technique (Maccario et al.

Results

Figure 2 shows the results of the analysis of X-chromosome inactivation by means of the pSPT-PGK probe in different cell types derived from the obligate carriers II,14 (Fig. 2A), I,2 (Fig. 2B), and II,8 (Fig. 2C), and Fig. 2A–D. Analysis of randomness of X-chromosome inactivation in different DNA preparations digested with EcoRI. BglI, BglII, in the absence ("-"), and in the presence ("+"), of HpaII, and hybridized with the pSPT-PGK probe. A Analysis in T cells (lanes 1, 2), lymphoblastoid B cell line (lanes 3, 4), neutrophils (lanes 5, 6) and fibroblasts (lanes 7, 8) from subject II,14. B Analysis in T cells (lanes 1, 2), lymphoblastoid B cell line (lanes 3, 4), and neutrophils (lanes 5, 6) from subject II,8. C Analysis in T cells (lanes 1, 2), lymphoblastoid B cell line (lanes 3, 4), and neutrophils (lanes 5, 6) from subject II,14. D Analysis in whole-blood-derived DNA from subjects II,4 (lanes 1, 2), II,5 (lanes 3, 4), and II,10 (lanes 5, 6)