Localization of the HLA class II-associated invariant chain gene to human chromosome band 5q32

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HLA class II antigens are present as heterodimers of α and β chains at the surface of B lymphocytes, macrophages, Langerhans cells, and a few other cell types involved in the immune response, including a subset of T lymphocytes (Cresswell 1987). Intracellularly, they are found in association with a third molecule that, in contrast to the highly polymorphic α and β chains, shows a constant electrophoretic mobility and has therefore been called γ (Kvist et al. 1982) or invariant (Jones et al. 1978) chain. The function of the HLA class II-associated invariant chain has not yet been elucidated, though it is speculated that it may play a role in facilitating assembly or intracellular transport of α and β chains (Cresswell 1987) or both. As the invariant chain presents several structural and biochemical similarities to the transferrin receptor, the parallel could extend to function as well, which suggests a putative role in antigen processing and presentation through endocytosis (Rosamond et al. 1987). As a rule, the expression of HLA class II α, β, and γ chains is coordinately regulated both in cell types normally expressing HLA class II antigens (Narni et al. 1987) and after γ-interferon stimulation (Paulnoch-King et al. 1985). While the α and β genes are clustered in the major histocompatibility complex region on the short arm of chromosome 6, the invariant chain has been assigned to chromosome 5 by somatic cell hybrid methodology (Claesson-Welsh et al. 1984). Here we report on its sub-localization to band 5q32 by in situ hybridization to metaphase chromosomes.

An invariant chain cDNA clone, p7-2D-1446 (Kudo et al. 1985), was digested with Pst I to release the 1.44 kb insert from pBR322. The insert was then radiolabeled by random priming (Feinberg and Vogelstein 1983) with [3H]dATP (42.3 Ci/mmol) and [3H]dTTP (93 Ci/mmol) (New England Nuclear, Boston, Massachusetts) to a specific activity of 1 × 10⁶ cpm/µg. Lymphocytes from two healthy male volunteers were cultured in the presence of phytohemagglutinin. Synchronization was achieved by adding 5-bromodeoxyuridine to a final concentration of 200 µg/ml after 72 h. Cells were then washed and reincubated for 6.5 h in the presence of 10⁻³ M thymidine. Slides were stored 7-14 days at room temperature before in situ hybridization experiments were performed. Hybridization was performed essentially as described by Harper and Saunders (1981). After ribonuclease-A treatment, chromosomal DNA was denatured and then hybridized to the 3H-labeled Pst I insert from p7-2D-1446 at a concentration of 10⁻⁴₀ ng/ml, followed by exposure in Kodak NTB2 nuclear track emulsion (Eastman Kodak, Rochester, New York) at -80 °C for 5-10 days and development in Kodak Dektol at 15 °C. Chromosomes were G-banded by the FPG technique (Perry and Wolff 1974).

In a first experiment the total distribution of labeled sites was recorded. Only silver grains overlapping or clearly touching chromosomes were considered for analysis. On a total of 100 labeled sites scored from 44 metaphases, 21 were localized on chromosome 5 (21%), confirming the previous assignment obtained through analysis of a mouse-human somatic cell hybrid panel (Claesson-Welsh et al. 1984). A significant clustering of grains (16%) was observed on the region 5q31-q34, while no other region displayed labeling above background (Fig. 1). A second experiment was performed using lymphocytes from a different donor to define more accurately the grain distribution on chromosome 5. In 35 copies of chromosome 5 analyzed, 27 grains (77%) were localized to bands 5q31–q34. Peak labeling was scored on band 5q32, with 16/35 (46%) silver grains touching this region (Fig. 2). A representative metaphase exhibiting labeling of 5q32 is shown in Figure 3. Based on these results, we have assigned the invariant chain gene to chromosome band 5q32.

The assignment of the HLA class II-associated invariant chain gene to band q32 of chromosome 5 is significant
in view of the fact that the long arm of chromosome 5 is frequently deleted in hematological disorders related to antileukemic treatment, such as therapy-related refractory anemia (t-RA) and acute nonlymphocytic leukemia (t-ANLL) (Le Beau et al. 1986a, Pedersen-Bjergaard and Philip 1987). Similar deletions are found in bone marrow cells from patients with primary refractory anemia — the so-called 5q− syndrome — and from a minority of patients with primary ANLL (Van den Berghe et al. 1985). A critical segment that is consistently absent from the deleted chromosome 5 has been identified cytogenetically in the region 5q23−31 (Van den Berghe et al. 1985, Le Beau et al. 1986a). The same region contains an impressive number of genes encoding growth factors and their receptors. Among these are some of the known colony-stimulating factors (CSFs) and their receptors, which are involved in proliferation and differentiation of hematopoietic cells. The genes coding for CSF-1 or M-CSF (macrophage-CSF), CSF-2 or GM-CSF (granulocyte-macrophage-CSF), and interleukin 3 and the fms proto-oncogene (which is presumed to be identical to the CSF-1 receptor) are all enclosed in the segment 5q23−33.1 and are frequently deleted in 5q− cells (Le Beau et al. 1986b, 1987, Pettenati et al. 1987). Other growth-related genes located in close proximity to these CSFs include endothelial cell growth factor (Jaye et al. 1986), which is closely related or possibly identical to acidic fibroblast growth factor (Lobb et al. 1986), and the receptor for platelet-derived growth factor (Yarden et al. 1986). This last one is also commonly deleted in 5q− cells (Le Beau et al. 1987). These findings have led to speculation that the pathogenesis of 5q− disorders might be analogous to that of retinoblastoma and Wilms’ tumor, where deletions of specific chromosome sequences are involved in activation of recessive oncogenes or inactivation of tumor suppressor genes (Knudson 1986). One or more growth-related genes mapped to the long arm of chromosome 5 could be involved in the pathogenesis of ANLL, and the different clinical and hematological picture could depend on differential involvement of these genes.