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Immunology

Allelic exclusion of immunoglobulin expression is not caused by somatic segregation

(IgD allotype/mitogenic B-cell stimulation/Robertsonian translocation on mouse chromosome 12)

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ABSTRACT **We have investigated the karyotype of immunoglobulin-producing cells in heterozygous animals. Using a karyotypic marker for one homolog of a chromosome carrying immunoglobulin genes, we established that immunoglobulin-producing cells are heterozygous with respect to this chromosome. Therefore, allelic exclusion of immunoglobulin expression cannot be caused by somatic chromosome segregation.**

A single lymphocyte produces identical immunoglobulin molecules with a single pair of variable regions for heavy and light chain to define its monospecificity. This state of affairs is not simply an empirical observation but is a necessary condition for clonal selection to occur. There are, *a priori*, two possibilities: either the identical variable regions are transcribed from both paternal and maternal chromosomes or a single cell expresses genes of either maternal or paternal chromosomes but not of both. The second possibility, termed allelic exclusion, has been shown to occur as a rule for mature B lymphocytes or plasma cells (1–5).

Studying metaphase chromosome spreads of spleen cells of the deer mouse, Ohno found somatic segregation for some autosomes in some of the metaphases. This observation led him to propose a theory of somatic segregation of the chromosomes carrying the genes for the immunoglobulin chains (6). As a consequence of this segregation, a heterozygous cell producing immunoglobulin chains should be homozygous with respect to the chromosomes carrying the genes for the immunoglobulin chains. In the mouse, these are chromosome 12 for the heavy chain (7, 8), 6 for the κ chain (7, 9), and 16 for the λ chain (10). A karyotypic marker on one homolog of these chromosomes would allow us to test for the homozygosity of these chromosomes in immunoglobulin-producing cells from heterozygous animals.

MATERIALS AND METHODS

(Rb5 × BALB/c)F_1 mice were provided by J. Johnson (Munich).

Spleen cells from (Rb5 × BALB/c)F_1 mice were cultured at an initial density of 10^6/ml in RPMI medium/20% fetal calf serum/50 μM 2-mercaptoethanol/25 mM Hepes containing lipopolysaccharide at 40 μg/ml. The cells were harvested for metaphase spreads on day 3 of culture. Metaphases were prepared and banded with Giemsa/trypsin stain by standard methods (11, 12). Immunofluorescence study was as described (13).

RESULTS AND DISCUSSION

The Rb5 mouse strain carries the Robertsonian translocation Rb(8;12)5Bnr (14). In (Rb5 × BALB/c)F_1 mice, the expected karyotype would display two morphologically different homologs of chromosome 12: one acrocentric and one metacentric chromosome being composed of chromosome 12 and 8. If somatic chromosome segregation were responsible for allelic exclusion, a B lymphocyte should contain either two metacentric or two acrocentric homologs of chromosome 12.

We first established that spleen B lymphocytes of the F_1 mice show allelic exclusion. Most of those cells have IgM and IgD on their surface (15). As the mice carry the immunoglobulin heavy chain gene clusters of *a* and *b* haplotype, we used monoclonal antibodies to the δ chain of *a* and *b* allotype [hybridoma lines 10.4.22 and 11.6.3 of Oi *et al.* (16)]. Of 2335 spleen cells examined by the double immunofluorescence technique (13), 9% expressed δ chains of *a* allotype and 7% expressed δ chains of *b* allotype. No cell expressed δ chains of both allotypes.

Lipopolysaccharide stimulates B lymphocytes to divide (17). We therefore studied the metaphase chromosomes in lipopolysaccharide-stimulated spleen cell cultures. If somatic chromosome segregation were responsible for allelic exclusion in the unstimulated spleen cells, the progenitors of those cells, under no circumstances, could revert to synthesis of immunoglobulins of both haplotypes. At the day of metaphase spreading, 63% of the lymphocytes produced membrane immunoglobulin. Of 5437 cells counted, 1.6% expressed δ chains of *a* allotype, and 1.3% expressed δ chains of *b* allotype. No cell expressed δ chains of both allotypes, indicating that lipopolysaccharide-stimulated B cells are allelically excluded. All 304 metaphases analyzed displayed heterozygosity with respect to chromosome 12 (Fig. 1). Therefore, we exclude somatic chromosome segregation as the mechanism for allelic exclusion.

Rearrangement of DNA may be productive or nonproductive on either homologous chromosome (18, 19). Recently, Nottenburg and Weissman (20) have shown that heterozygous cells expressing immunoglobulin heavy chains of only one haplotype contain the genes for the constant region of the μ chain of both haplotypes. This analysis of DNA complements our cytogenetic study and excludes the somatic segregation theory of allelic exclusion. Nevertheless, Ohno's observation of somatic segregation in the deer mouse remains to be explained.

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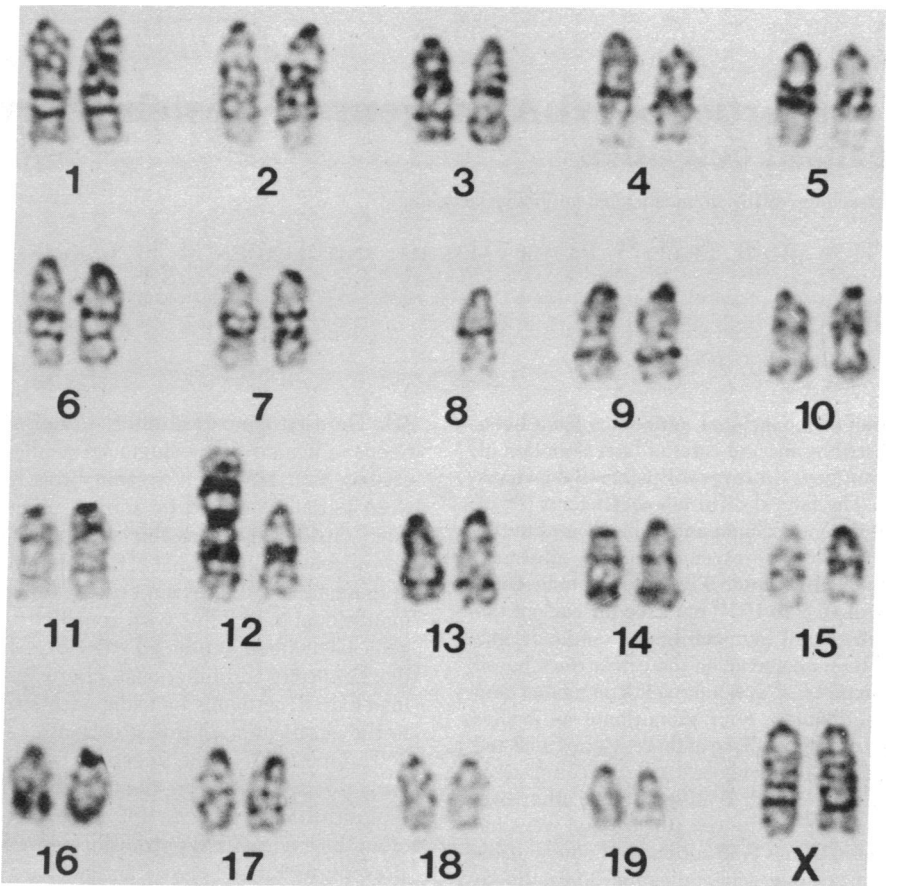

FIG. 1. Karyotype of a B lymphocyte from an (Rb5 × BALB/c)F_1 mouse.

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