Brief Definitive Report

aIr-1, A NEWLY FOUND LOCUS ON MOUSE CHROMOSOME 16 ENCODING A *TRANS*-ACTING ACTIVATOR FACTOR FOR MHC CLASS II GENE EXPRESSION

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The MHC-encoded class II or Ia antigens are highly polymorphic glycoproteins consisting of a heterodimeric structure with an α subunit of 33–36,000 kD and a β subunit of 24–28,000 kD (1). They play an important role in the homeostasis of the immune system (2).

In man there are at least three class II genetic subregions designated DP, DQ, DR, each encoding distinct α/β heterodimers (1, 3, 4).

Control of Ia antigen expression at the cell surface, in addition to polymorphism and heterogeneity, influences the role of class II antigens in the regulation of the immune response (5).

We recently described a human B cell variant, RJ 2.2.5, derived from Raji cells after mutagenesis and immunoselection with anti-Ia mAb (6). The RJ 2.2.5 cells express none of the class II antigens (6), as shown by a lack of specific class II mRNAs (7, 8). The class II–positive phenotype of RJ 2.2.5 cells can be restored in somatic cell hybrids between this variant and mouse B lymphoma cells (8) or mouse spleen cells (9). Phenotypic and structural studies have indicated that in these hybrids a mouse factor, unlinked to MHC products, is acting as an activator that positively regulates the expression of both mouse and man class II genes (9). Karyotypic analysis and segregation studies in an informative family of hybrids have now permitted us to assign the locus of this *trans*-acting activator for class II genes to mouse chromosome 16.

Materials and Methods

Somatic Cell Hybrids. In previous reports, we have described the derivation of the RJ 2.2.5 Ia^- variant (6) and of RJ 2.2.5 × mouse spleen cell hybrids (9) used in this study. Selection of the hybrids on the basis of the presence or absence of a relevant MHC antigen on the cell surface was carried out on a FACS II (Becton Dickinson Immunocytometry Systems, Mountain View, Ca) after indirect immunofluorescence staining of the cells with specific mAbs (see legend to Fig. 1), as reported (9).

Karyotype Analysis. The karyotype of the various cell lines described in this study was analyzed after metaphase chromosome preparation and staining by G-banding following standard procedures.

DNA Analysis. Genomic DNA from the various cell lines was extracted as described

previously (8). After digestion with Hind III, the DNA was separated on a 0.7% agarose gel, transferred to Gene Screen Plus hybridization transfer membranes (Amersham Corp., Buckinghamshire, United Kingdom), and hybridized with an α-[^{32}P]dNTP-labeled mouse Vλ1 probe, pc Vλ1, derived from the genomic Vλ1 clone described in reference 11, and kindly donated by Dr. S. Weiss (Basel Institute for Immunology, Switzerland). The filters were washed in stringent conditions according to the specification of the manufacturers, and they were exposed to X-OMAT Kodak films for 10 d at −80°C.

Results and Discussion

To assign the locus encoding the *trans*-acting factor (activator of immune response genes–locus 1 [*aIr-1*]) to a specific mouse chromosome we selected a series of RJ 2.2.5 × mouse spleen cell hybrids (9) with specified phenotypic characteristics. In one clone, designated A2-2.4 c.6 (similar to clone c.9 in reference 9), human HLA class I (A,B,C) antigens were expressed in 100% of the cells; human class II antigens of the DR subset were expressed in 90% of the cells. In a second class II subset, the DQ antigen was expressed in 87% of the cells (data not shown). Mouse H-2 class I (H-2K) and class II (I-A) antigens were expressed in 50% of the cells (see Fig. 1*A*).

A2-2.4 c.6 cells had a modal chromosome number of 93–94. A pseudotetraploidy of the human genome was found, with a number of chromosomes ranging between 91 and 93. The parental RJ 2.2.5 human Ia$^-$ cells presented a chromosome number of 46, with various chromosome abnormalities including t(8;14) and trisomy 7 (data not shown), frequently found in human Burkitt lymphoma lines (10). The human pseudotetraploidy of A2.2-4 c.6 probably arose by an accidental tri-parental mating, as suggested by the fact that all clones derived from the hybrid A2 exhibited similar human chromosome complement. Analysis of a large number of metaphases of A2-2.4 c.6 cells (see Fig. 1) revealed the presence of two mouse chromosomes, namely the 17 and a second chromosome, which by cytogenetic characteristics was identical to mouse chromosome 16 except for a small deletion of part of its distal region (band C3 and probably part of C2 and C4) (see Fig. 1 *B*, *top*). The assignment was confirmed by Southern blot hybridization with a probe specific for the Vλ1 Ig gene (11) located on mouse chromosome 16 (12, see below). Karyotyping of A2-2.4 c.6 cells showed that mouse chromosome 17 was present in 50% of the metaphases analyzed, in agreement with the proportion of cells actually displaying mouse MHC class I antigens on the cell surface. Mouse chromosome 16 was found in 86% of the metaphases analyzed, in agreement with the proportion of cells reexpressing human class II antigens in the clone hybrid. These results suggested that the presence of mouse chromosome 16 correlated with the presence of the class II gene-activator *trans*-acting factor. To better demonstrate this point, we used the FACS to separate the A2-2.4 c.6 population into two fractions on the basis of a DR$^+$ and DR$^-$ phenotype, respectively. The cells from each fraction were first expanded by in vitro culture and then karyotyped. The results of such analysis are reported in Fig. 1. At the time of karyotyping the DR$^+$-selected cells expressed the following cell surface MHC phenotypes: HLA, A,B,C, 100%; DR, 91%; H-2K, 23%; I-A, 25%. Accordingly, mouse chromosome 16 was present in 92% and mouse chromosome 17 in 24% of the metaphases analyzed. DR$^-$-selected cells expressed the following cell surface MHC phenotypes: HLA A,B,C, 100%; DR, 2%; H-2K, 13%. Accordingly, mouse chromosome 16 was present in 3%

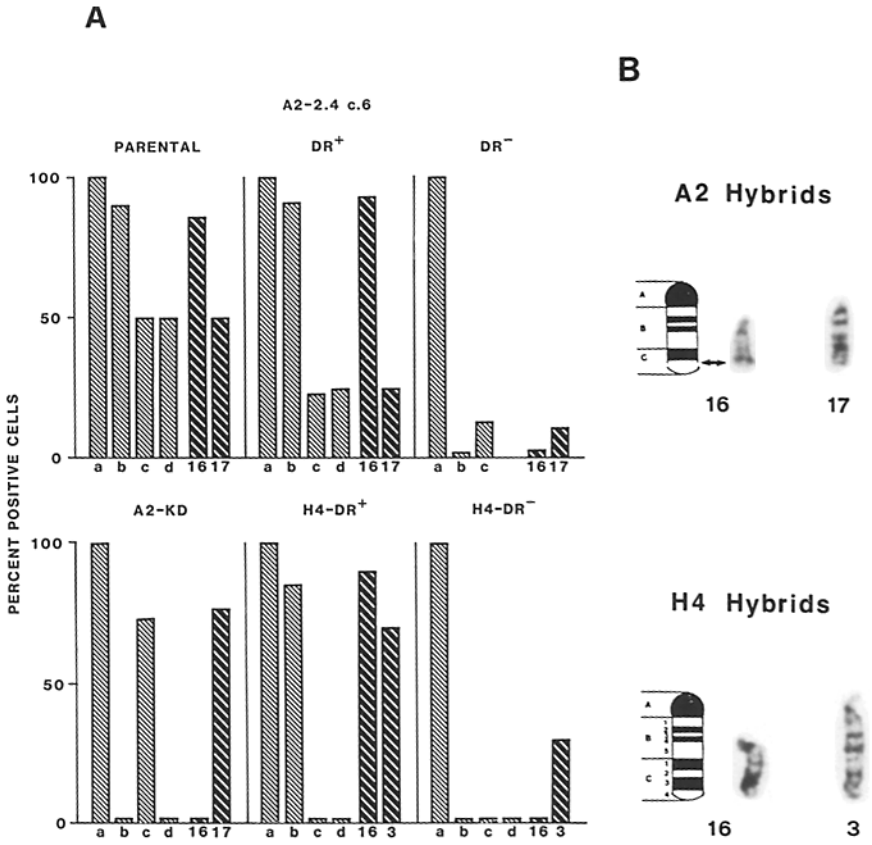

FIGURE 1. (*A*) MHC phenotype and corresponding mouse chromosomes found in informative RJ 2.2.5 × mouse spleen cell hybrids. The MHC phenotype of the hybrids listed on the top of each panel was assessed by indirect immunofluorescence with specific mAbs and FACS. (*a*) HLA-A,B,C (mAb B9.12.1 [14]); (*b*) DR (mAb D1–12 [3]); (*c*) H-2K (mAb S13.11 [15]); (*d*) I-A (mAb 25.9.17 [16]). Results are expressed as percent positive cells (ordinate). For each hybrid 40–60 metaphases were analyzed and the number of metaphases with the specific mouse chromosomes (16, 17, and 3) listed on the abscissa was expressed as percent of the total number analyzed (ordinate). (*B*) Aspect of metaphase mouse chromosomes 16, 17, and 3 (G-banding) as found in the various hybrids analyzed in this study. When present (see *A*), the chromosome 16 in A2-derived hybrids (*upper panel*) presented a small deletion (*arrow*) of the distal region (band C3, and probably part of C2 and C4), in contrast to the canonic chromosome 16 found in H4-derived hybrids (*lower panel*). A schematic representation of mouse chromosome 16 is also reported in both panels for comparison.

and mouse chromosome 17 in 11% of the metaphases analyzed. These results were further corroborated by DNA analysis of the two distinct hybrid populations. Hybridization with the mouse Vλ1 probe was clearly positive with the DR^+-selected cells (Fig. 2*b*), whereas no hybridization was observed with the DR^--selected cells (Fig. 2*c*). Therefore, segregation of mouse chromosome 16 was accompanied by the loss of reexpressibility of human class II genes in the A2-2.4 c.6 hybrid.

The association of mouse chromosome 16 with the reexpressibility of the human class II genes was further documented by the analysis of two other clones, one of which, A2-KD, was derived from the same original A2 hybrid (9) after selection for $H\text{-}2K^+$ cells and further cloning by limiting dilution technique; the

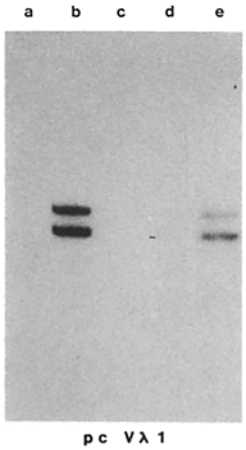

FIGURE 2. Presence of a mouse chromosome 16 marker in various hybrids and parental cell lines as detected by Southern blot hybridization with an Ig Vλ1 probe. (*a*) RJ 2.2.5 DNA; (*b*) DNA from a FACS-enriched DR^+ population from A2-2.4 c.6 presenting chromosome 16 in its karyotype; (c) DNA from a FACS-enriched DR^- population from A2-2.4 c.6 not containing chromosome 16 in its karyotype; (*d*) DNA from A2-KD, a DR^- derivative of A2; (*e*) DNA from M12.4.1, a mouse B cell lymphoma line.

second clone, designated H4, originated from a distinct hybrid selected early after fusion of RJ 2.2.5 and mouse spleen cells. Interestingly, the A2-KD clone did not express either human or mouse class II gene products, although human class I was expressed in 100% of the cells and mouse H-2K in 74% of the cells (Fig. 1*A*).

Karyotype analysis of A2-KD clone revealed the presence of a single mouse chromosome, namely chromosome 17 (Fig. 1, *A* and *B*), in 76% of the cells; it also revealed the pseudotetraploidy of human chromosomes already observed for the A2-2.4 c.6 hybrid cells. As expected, Southern blot hybridization experiments revealed no hybridization of the probe containing the Vλ1 gene with DNA prepared from A2-KD cloned cells (Fig. 2 *d*).

The second hybrid, namely H4, displayed an MHC cell surface phenotype consisting of: HLA-A,B,C, 100%; DR, 42%; H-2K, 1%; I-A, 1% (data not shown). As for A2.2-4 c.6 hybrid, H4 cells were selected with the FACS on the basis of DR^+ and DR^- phenotype, and then karyotyped. Interestingly, both H4 populations had the same human ploidy as RJ 2.2.5 cells (46 chromosomes, data not shown). Two mouse chromosomes were detectable by karyotyping, the 16 and the 3 (Fig. 1, *A* and *B, bottom*). In contrast to A2-derived hybrids, a canonic mouse chromosome 16 was present in the H4 hybrid. The presence of mouse chromosome 3 correlated with the ouabain-resistance selection (13) originally adopted to select for hybrids after RJ 2.2.5 × mouse spleen cell fusion (9). In the DR^+ (85%) population, chromosome 16 was present in 89% and chromosome 3 in 70% of the metaphases analyzed. In the DR^- population, chromosome 16 was never found, whereas chromosome 3 was found in 30% of the metaphases analyzed (Fig. 1*A*). These results confirmed the exclusive association of the human Ia^+ phenotype with the presence of mouse chromosome 16.

Taken together, the results presented in this report show that the presence of a single copy of mouse chromosome 16 in RJ 2.2.5 × mouse spleen cell hybrids

is a necessary and sufficient condition to allow expression of both mouse and human class II genes. We establish the existence of a newly found locus, *aIr-1*, that is located in this chromosome and determines a *trans*-acting activator function. Within this frame, the class II–negative phenotype in RJ 2.2.5 cells may thus be interpreted as originating from a lesion in the human equivalent of the *aIr-1* locus coding for, or affecting the expression of, the *trans*-acting regulatory factor.

The fact that a recessive lesion in a seemingly autosomal locus could be expressed in RJ 2.2.5 cells suggests either that these cells carry a double mutation at the *aIr-1* locus-equivalent or that only one allele of this regulatory gene is normally functional in B cells. In the latter case, the other copy of the gene might be kept silent by allelic exclusion or a similar mechanism. In the former case, the two mutations could be cogenerated at the same time when Raji cells were mutagenized to obtain the RJ 2.2.5 variant (6), or alternatively, Raji cells used for mutagenesis could contain only one functional copy of the regulatory locus because of a previous independent mutational event that removed or inactivated one of the two alleles. The occurrence of chromosomal rearrangements in Burkitt lymphomas (10) may have some bearing on this point. The possibility that a gene dosage mechanism may be responsible for the phenotype of RJ 2.2.5 cells seems less likely in view of the fact that, as proven by karyotype analysis, one copy of chromosome 16 was sufficient to allow reexpression of both mouse and human class II genes.

The discovery of the *aIr-1* locus and its assignment to mouse chromosome 16 will stimulate further efforts to isolate and clone the corresponding gene. This will in turn permit a biochemical characterization of the gene product and a detailed analysis of its mode of action. Moreover, the assessment of the role of this *trans*-acting factor in the developmental regulation of the expression of class II genes and of its influence on the functions mediated by class II gene products will be greatly facilitated.

Summary

RJ 2.2.5 is a human B cell line that has lost the capacity to express MHC class II genes. The human class II–positive phenotype is restored in somatic cell hybrids between RJ 2.2.5 and mouse spleen cells.

By karyotype and molecular studies of an informative family of hybrids we have now shown that the reexpression of human class II gene products, as well as the maintenance of the mouse class II–positive phenotype, correlates with the presence of mouse chromosome 16. Thus, the existence on this mouse chromosome of a newly found locus, designated by us *aIr-1*, that determines a *trans*-acting activator function for class II gene expression, is established. Possible implications of this finding are discussed.

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