*Brief Definitive Report*

# Reactivation of a Major Histocompatibility Complex Class II Gene in Mouse Plasmacytoma Cells and Mouse T Cells

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## Summary

Terminally differentiated plasma cells and mouse T cells do not express major histocompatibility complex (MHC) class II genes although class II gene expression is observed in pre-B and mature B cells as well as in activated human T cells. Transient heterokaryons were prepared and analyzed to investigate the mechanisms of inactivation of MHC class II gene in mouse plasmacytoma cells and mouse T cells. The endogenous MHC class II genes in both mouse plasmacytoma cells and mouse T cells can be reactivated by factors present in B cells. This reactivation of class II gene is also observed by fusion with a human T cell line which expresses MHC class II genes, but not with a class II negative human T cell line. It appears that the loss of MHC class II gene expression during the terminal differentiation of B cells or T cell lineage is due to absence of positive regulatory factor(s) necessary for class II transcription.

MHC-encoded class II antigens are heterodimeric cell surface molecules that present antigens to CD4 positive lymphocytes and thus play an important role in the regulation of immune responses. The expression of MHC class II molecules is developmentally regulated, and only limited cell types normally express class II antigen, primarily APCs such as macrophages, dendritic cells, and B cells. Class II expression can be induced on human T cells by mitogens or antigens (1–3) and on macrophages, fibroblasts, and endothelial cells by IFN-$\gamma$ (4).

The molecular mechanisms responsible for the regulation of MHC class II gene expression in B cells have been extensively studied (for review see reference 5). Many *cis* regulatory elements have been identified, and DNA binding proteins which could potentially be involved in the regulation of class II gene expression have been characterized. Constitutive expression of class II genes on B cells is lost during terminal differentiation into plasma cells. Consistent with this idea, fusion between mouse plasmacytoma and human B cells resulted in a class II negative hybrid. The extinction of class II gene expression was thought to have resulted from the presence of dominant suppressor factors in plasmacytoma cells (6, 7). A similar suppressive effect was observed in a hybrid between the class II negative mouse T lymphoma BW5147 and class II positive B cell blasts (8). However, these studies were carried out using stable lines derived by cell fusion. A major problem with this approach is the potential to lose chromosomes during the large number of generations of cell growth required to obtain the hybrids. To overcome this problem, the transient heterokaryon assay was introduced (9). Cell fusion of heterologous cell lines produces transient heterokaryons in which the nuclei of the parental cell types remain separate but are exposed to diffusible regulatory factors from the cytoplasm and nucleus of each cell type. This approach, therefore, provides a direct and immediate assay for tissue- and stage-specific *trans*-acting regulatory factor(s). Studies that used heterokaryons have yielded evidence for the involvement of positive *trans*-acting factors in regulation of cell type-specific expression of variety of genes (9–11). It is surprising that we show here that a mouse MHC class II gene can be reactivated in both mouse plasmacytoma and T cells upon fusion with MHC class II positive cells.

## Materials and Methods

*Cell Culture.* The mouse plasmacytoma cell line MPC11 and human T cell line Jurkat were purchased from American Type Culture Collection (Rockville, MD). Mouse plasmacytoma cell line P3X63-Ag8.563 was obtained from the hybridoma facility at Yale Medical School. The mouse plasmacytoma cell line P3U1 and human B cell line Raji were obtained from Dr. R. Accolla (Istituto di scienze immunologiche, Rome, Italy). The human T cell line H-9 was a gift from Dr. N. Ruddle (Yale University) and the mouse T cell line BW5147 was obtained from Dr. C. Janeway (Yale University). All cell lines used are maintained in RPMI supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine. Mouse spleen cells were prepared from C57B6/J mice.

*Cell Fusion.* For most experiments, $2 \times 10^7$ cells of each cell line were mixed, passed through an 18-gauge syringe needle to

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disaggregate cells and plated on poly-L-lysine–treated dishes. After 2 h, the culture medium was aspirated and the plates were washed with prewarmed serum-free medium. 2 ml of prewarmed polyethylene glycol (PEG) 1000 (Koch-Light, Ltd., Hatfield, UK) (50% [wt/vol] serum-free DMEM; pH adjusted to ~7.4 by the addition of 7.5% $NaHCO_3$) were added and incubated at 37°C for 90 s. The plates were then washed gently three times with prewarmed serum-free medium, and incubated in medium containing 10% FBS for 2 d. Cytoplasmic RNA was prepared as described before (12).

*RNA Analysis.* RNase protection assays were described previously (13). Probe for Eα and actin was described before (13).

*PCR Analysis.* First strand cDNA was synthesized by reverse transcriptase (Seikagaku America Inc., Rockville, MD) using 10 ng of oligo dT as a primer and 2 μg of cytoplasmic RNA in 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin, 1 mM DTT, 200 μM dideoxynucleotides. PCR amplification was performed using 10% of synthesized first strand cDNA, 5 U of Taq DNA polymerase (Perkin-Elmer Cetus Corp., Norwalk, CT) in the reaction condition, as was done for first strand synthesis. 30 cycles were run for 1 min at 94°C, 1 min at 55°C, and 3 min at 72°C. The sequences of oligonucleotide primers used in PCR amplification are as follows: for sense sequence CCAGAAGTCATGGGCTATCA in the exon 1, and for non-sense sequence GGCTCCTTGTCGGCGTTCTA in exon 4.

## Results and Discussion

*Activation of MHC Class II Gene Expression in Mouse Plasmacytoma Cells after Fusion with MHC Class II Positive Cells.* To distinguish gene expression of the plasmacytoma cell from the B cell fusion partner, we used cell lines of different species. Transient heterokaryons were generated using mouse plasmacytoma cells MPC11 and the human Raji B cell lymphoblastoid cell line. When RNA from an unfused, mixed population of MPC11 plus Raji cells was analyzed with a mouse MHC class II Eα gene probe, a protected fragment was not observed (Fig. 1, lane *3*). However, when RNA obtained from MPC11 × Raji heterokaryons 48 h after fusion was analyzed, Eα transcripts were clearly present (Fig. 1, lane *4*). Similar results were obtained irrespective of the cell ratio used in fusion (cell ratio of either 1:5 or 5:1, data not shown). To determine whether this reactivation of the MHC class II gene was restricted to a specific plasmacytoma cell line, MPC11 cells, we also tested two additional mouse plasmacytoma cell lines, Ag8 and P3U1. The Eα gene was also reactivated in these cells by fusion with human B lymphoblastoid cells, although the levels of transcripts varies somewhat between different cell lines (Fig. 1, lanes 5–8). The P3U1 plasmacytoma line was used for the original study by stable cell hybrids which suggested that P3U1 cells contain a dominant suppressor factor that inhibits the expression of MHC class II gene (6).

We also used different cell types as partners for the heterokaryons to determine whether only human B cells can complement the activation of the silent mouse MHC class II gene. Plasmacytoma cells were fused with two different human T cells lines, H-9 and Jurkat cells, which are MHC class II positive and negative, respectively. As shown in Fig. 2, Eα transcripts were detected in RNA from fused MPC11 × H-9 cells but not mixed cells (Fig. 2, lanes *3* and *4*). In contrast,

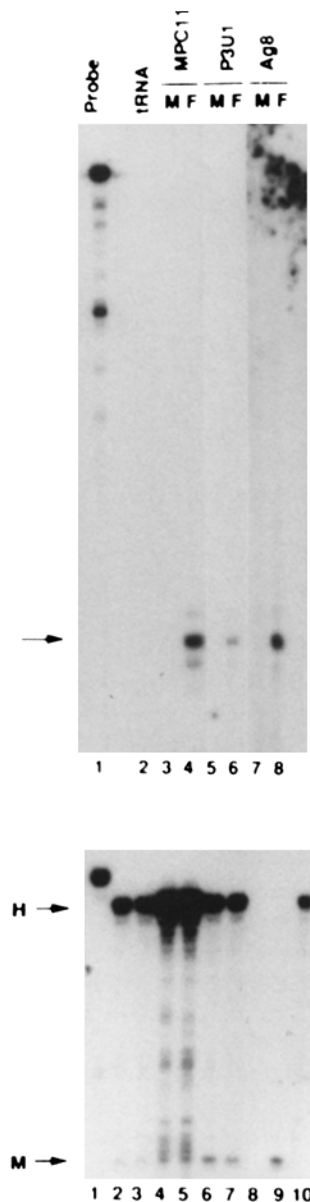
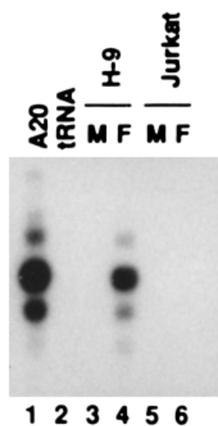



**Figure 1.** Activation of Eα gene in plasmacytoma cells, MPC11, after fusion with human B lymphoblastoid cells (Raji cells). (*Top*) RNase protection assay using 50 μg of RNA hybridized with Eα probe. (*M* and *F*) Mixed and fused, respectively. (*Arrows*) Protected signals. (*Bottom*) Actin control. 5 μg of RNA were hybridized with human actin probe which also hybridizes mouse actin. Lanes: *1*, probe; *2–7* correspond with *3–8* (*top*); *8*, tRNA; *9*, mouse actin control; and *10*, human actin control.

Jurkat cells that do not express MHC class II genes cannot reactivate Eα expression in MPC11 cells upon fusion (Fig. 2, lanes *5* and *6*). Again, the activation of the mouse MHC class gene which is normally transcriptionally silent in MPC11 cells is likely because of the presence of positive *trans*-acting regulatory factors supplied by class II positive cells.

A cell sorting experiment was performed to confirm if the reactivation of class II gene is restricted to cells fused between two different cell types and not present in either homotypic



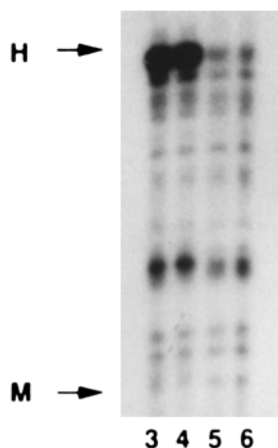




**Figure 2.** Activation of Eα gene in plasmacytoma cells, MPC11, by human T cells (H-9 and Jurkat cells). Assay condition is the same as described in legend to Fig. 1. A20 cells are mouse B lymphoblastoid cells and 10 μg of A20 RNA were used. *(Bottom)* Actin control. Lane numbers correspond.

fused cells or unfused cells. Fused cells between two different cell types can be isolated from the parental cells using different cell surface markers in mouse and human cells such as MHC class I molecule. RNA from three different populations of cells, human, mouse, and fused cells between mouse and human cells, were prepared and analyzed after sorting. Only heterotypic fused cells express both mouse and human class II gene (data not shown).

*Activation of the Mouse Eα Gene in Mouse T Cells after Fusion with Human B and T Cells.* Although human T cells express MHC class II genes upon activation, mouse T cells do not express mouse class II genes. To determine whether the MHC class II gene, Eα, can be reactivated in mouse T cells, we examined the expression of the Eα gene after fusion of the mouse T cell line BW5147 with human B or T cells. Before fusion, we were unable to detect Eα transcripts (Fig. 3, lanes *3* and *5*). However, after fusion of BW5147 cells with Raji or H-9 cells, accurately initiated transcripts were observed (Fig. 3, lanes *4* and *6*). Again, Jurkat cells that do not express MHC class II failed to complement mouse class II gene expression (Fig. 3, lanes 7 and *8*). Therefore, the MHC class II gene in mouse T cells is also reactivated by fusion with cells that express an MHC class II gene.

*Reactivation of Eα Gene Expression by Spleen Cells.* We also prepared C57/BL6 mouse spleen cells and used them as a

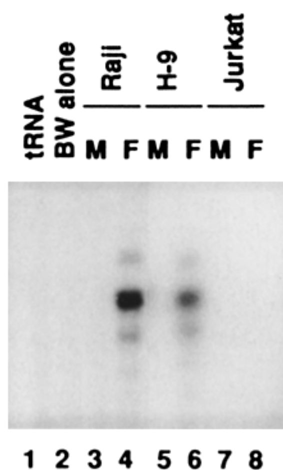



**Figure 3.** Activation of Eα gene in mouse T cells, BW5147, by human B lymphoblastoid cells (Raji cells) and human T cells (H-9 and Jurkat cells). Assay condition is the same as described in legend to Fig. 1.

fusion partner for both MPC11 and BW5147 cells to test whether normal B cells can also reactivate MHC class II expression in these cells. The Eα gene in this mouse strain has a deletion in the promoter and part of this first exon such that the Eα gene is not expressed (14, 15). Furthermore, since the volume of splenic B cells is an order of magnitude smaller than that of an established B lymphoblastoid cell line (5–7 vs 15–25 μm in diameter), if Eα expression was observed upon fusion using spleen cells, we could rule out the possibility that a hypothetical plasmacytoma or T cell negative regulatory factor(s) could be diluted out because of the approximately twofold difference in size of the original cell and the heterokaryon. Since the fusion efficiency of spleen cells is lower (4–5 vs. 10–15% determined by staining for MHC class I molecules) and about 60% of the total spleen cell population is B cells, we used PCR to detect Eα mRNA which

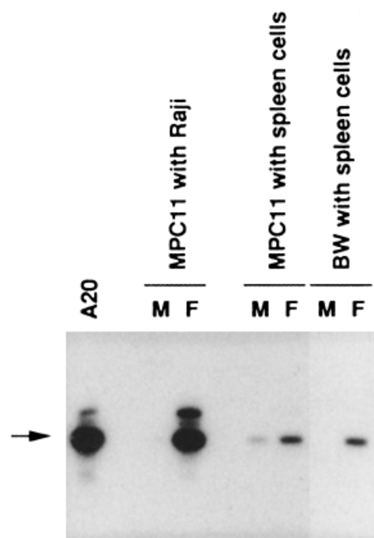



**Figure 4.** Activation of Eα gene by spleen cells. All lanes contain 10% of PCR product except A20 and MPC11 × Raji lanes which have 1% of PCR product. Lanes for BW5147 cells were exposed seven times longer than the other lanes.

is more sensitive than the RNase protection assay. Indeed, when RNA prepared from these fusions were analyzed using the RNase protection assay, we could not detect E$\alpha$ transcript (data not shown). However, as shown in Fig. 4 both MPC11 and BW5147 cells express E$\alpha$ upon fusion with spleen cells, although the level of transcripts is considerably lower than that seen in fusions with Raji cells. Low levels of E$\alpha$ transcripts are detected by PCR in MPC11 cells before fusion which we could not detect by the RNase protection assay. Mouse T cells, however, do not exhibit any transcripts unless they are fused with spleen cells. It seems therefore that at least some cell lines derived from the B cell lineage contain residual E$\alpha$ transcripts. We believe that the lower level of transcripts detected in fusions with plasmacytoma and normal spleen cells is a direct reflection of the small volume of the spleen cells with which the plasmacytoma cells were fused, and the reduced efficiency of fusion. We presume that the reduced level of complementation in these heterokaryons is a consequence of a suboptimal level of transactivator required for activation of the class II genes.

It has been suggested that the loss of MHC class II expression either in mouse plasmacytoma cells or mouse T cells is due to the expression of a dominant negative regulatory factor(s) which represses class II expression in these cells (6). Since reexpression of MHC class II gene occurs in our transient fusion studies, we believe that the absence of MHC class II is caused by the loss of positive regulatory factor(s) upon differentiation of these cells. The reactivation that we observe is unlikely to be explained by dilution of a negative factor since any given heterokaryon should only have the equivalent of a twofold change in volume; even if many cells fuse, probabilistic arguments state that most heterokaryons will have similar contribution from each partner. Moreover, reactivation can be obtained by fusion with splenic B cells, which results in a negligible volume increase. The fact that splenic B cell complementation is suboptimal is, however, consistent with a possible reduced contribution of positive factors from splenic B cells compared with the larger B lymphoblastoid cells. The reexpression of class II genes has also been obtained by fusion of mutant cell lines that are class II negative including those from Bare Lymphocyte Syndrome (BLS) patients who lack MHC class II expression (10). In one of the in vitro mutants (RJ 2.2.5) reactivation has also been achieved by the stable integration of mouse genomic sequences into the genome of the mutant cell line after DNA-mediated gene transfer (16). Therefore, a *trans*-acting factor provided either by the fusion partner or transfected genomic DNA permits reexpression of the class II genes in the mutants cells.

Gel mobility shift assays might determine whether there is any difference in DNA binding profile in the class II promoter between class II positive and negative cells. However, the promoter occupancy in vivo in various class II negative mutants does not entirely correlate with the pattern of binding found in in vitro gel mobility shift assays (17). In addition, in vivo footprinting data shows that most class II negative mutants have promoters unoccupied by these factors except for one mutant cell line, RJ 2.2.6, which shows no difference from class II positive cells (17). The E$\alpha$ promoter in several plasmacytoma cells is not occupied or is only minimally occupied by DNA binding proteins when studied by in vivo footprinting (Kara, C. J., and L. H. Glimcher, manuscript submitted for publication). We also have observed variable patterns of DNA binding for the X box and X2 binding proteins by comparing different plasmacytoma cell lines with B cell lines by gel mobility shift assay, but no clear correlation with the absence of expression is seen (Fador, W., C.-H. Chang, and R. A. Flavell, unpublished data). The transcriptional defect in these cells may therefore not be in the proteins detectable by this assay.

Our data suggest that the lack of class II expression in both the mutant cell lines and the plasmacytoma cells and mouse T cells studied here is due to the loss of positive regulatory factor(s). One model which is consistent with the data from both stable hybrids and transient heterokaryons is that plasmacytoma cells synthesize a repressor of the synthesis of a *trans*-acting factor(s) which shuts off the synthesis of positive regulatory factors. In that case, transient heterokaryons would contain sufficient positive transcription factors to reactivate the class II gene. If these factors had a long $t_{1/2}$, the repression of their synthesis would not affect the results in the transient assays. The identification of the positive regulatory factor(s) that is responsible for the reactivation of class II gene in plasmacytoma cells and T cells should give a better insight into our understanding of the regulation of class II gene expression.

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