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CIITA B-cell-specific promoter suppression in MHC class II-silenced cell hybrids

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Abstract In this study, various sets of somatic cell hybrids, generated by the fusion of epithelial cell lines with B-lymphoblastoid cell lines, were analyzed for the expression of major histocompatibility complex (MHC) class II antigens. We first demonstrate, in human and mouse intraspecies hybrids, the coordinate suppression of MHC class II, *Ii* (invariant chain) and *HLA-DM* gene transcription, and the release of the silencing by the addition of interferon gamma. Using interspecies hybrids, the segregation of human chromosomes allowed us to establish that MHC class II extinction is linked to the presence in the hybrids of the chromosomes from the epithelial fusion partner. Moreover, our data provide evidence that the expression pattern of MHC class II mRNA is correlated with that of the class II transactivator (CIITA), suggesting that CIITA is the actual target of the silencing. To gain further insight into the suppression phenomenon we performed luciferase assays which show that silencing affects the activity of the B-cell-specific promoter of *CIITA*. These results therefore demonstrate that the MHC class II gene silencing in somatic cell hybrids is due to an active suppression of one of the promoters of the *CIITA* gene, mediated by the epithelial cell fusion partner.

Key words Silencing · MHC class II · CIITA · Somatic cell hybrid

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Introduction

Major histocompatibility complex (MHC) class II molecules are essential for the presentation of antigens to T-helper lymphocytes which leads to the onset of a specific immune response directed against the antigen (Germain 1994). Cells of the immune system, such as B lymphocytes, dendritic cells, or macrophages, constitutively express the MHC class II glycoproteins. However, non-professional APCs, like epithelial or fibroblast cells, do not express the MHC class II molecules unless incubated with IFNγ (Glimcher and Kara 1992). This tissue-specific pattern of expression is detected for the different MHC class II isotype genes, in addition to *Ii* (invariant chain) (Collins et al. 1984), and two non-classical *HLA* genes, e.g., *HLA-DMA* and *HLA-DMB* (Kelly et al. 1991), which are also involved in antigen presentation (Pieters 1997).

MHC class II expression is essentially regulated at the transcriptional level (Boss 1997; Glimcher and Kara 1992). The analysis of cell lines defective for the expression of MHC class II antigens has led to the identification of different factors involved in this transcriptional control, namely RFX5 (Steimle et al. 1995), RFXAP (RFX-Associated Protein) (Durand et al. 1997), and CIITA (Class II TransActivator) (Steimle et al. 1993). Recent data show that CIITA, a non-DNA-binding protein (Steimle et al. 1993), interacts with the RFX5 transcription factor (Scholl et al. 1997) and different TBP-associated proteins (Fontes et al. 1997; Mahanta et al. 1997). These protein-protein interactions, as well as the presence of additional transcription factor complexes (Boss 1997), are required for a proper transcription initiation of the MHC class II genes.

The tissue-specific expression pattern of *CIITA* and MHC class II genes are similar, except that the transcription of *CIITA* precedes that of the MHC class II in non-professional APCs induced by IFNγ (Steimle et al. 1994). Moreover, cell lines established from immunodeficient patients belonging to the BLS complementation group A (Bontron et al. 1997; Steimle et al. 1993),

or *CIITA* knock-out mice (Chang et al. 1996), fail to express MHC class II, and, to a lesser extent, Ii and HLA-DMs. Taken as a whole, CIITA appears as the key molecule controlling the tissue-specific expression pattern of the MHC class II glycoproteins.

An insight into tissue-specific gene expression has been gained through the study of somatic cell hybrids generated by the fusion of cell lines from different tissular origins (Gourdeau and Fournier 1990). Such analyses aim towards the determination of putative dominant *trans*–acting effects, and of the parental cell line producing the *trans*–acting factor(s). In rare cases, these studies have even led to the identification of the locus(i) involved (Gourdeau and Fournier 1990).

The silencing of MHC class II expression observed during the differentiation of mature B lymphocytes into plasmocytes (Halper et al. 1978) has been studied by this approach: fusion experiments between plasmocytes and mature B cells have shown that the resulting somatic cell hybrids were not expressing the MHC class II genes, and that the parental plasmocyte cell line was providing the suppression factor (Dellabona et al. 1989; Latron et al. 1988). Recently, additional data assessed that the MHC class II silencing observed in these hybrids was mediated by the transcriptional repression of *CIITA* (Sartoris et al. 1996).

The mechanisms controlling the differential MHC class II patterns of expression observed in B-LCLs and in epithelial cell lines are not yet established. Previous data had established that MHC class II suppression was observed with hybrids generated either by the fusion of a murine B lymphoma with the L-929 fibrosarcoma (Stuart et al. 1989), or by the fusion of a mouse carcinoma cell line with different human B-LCLs (Bono et al. 1991). In each case, however, the silencing process can be relieved by treatment with IFNγ, which thereby reproduces the expression pattern of MHC class II genes in epithelial cells. Our objective was therefore to gain a further insight into the suppression mechanism through the analysis of intraspecies and interspecies somatic cell hybrids generated by the fusion of B-LCLs and epithelial cells. In this report, we provide evidence that MHC class II expression is suppressed by a *trans*–acting silencer(s) produced by the epithelial parental cell line. In addition, we show that the coordinate suppression of the MHC class II, *Ii* and *HLA-DM* gene expression is mediated by the silencing of the *CIITA* gene itself, through the lack of transcription from its B-cell-specific promoter.

Materials and methods

Cell culture and IFNs

The following cell lines (ATCC) were used: Daudi, a human B-LCL originating from a Burkitt lymphoma; A20, a mouse B cell lymphoma; HeLa, a human epitheloid cell line; KG1, a human monocyte-macrophage cell line; L(tk$^-$), a mouse fibroblast cell line; RAG, a murine adenocarcinoma cell line.The Daudi, KG1 and A20 cell lines were grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS), antibiotics, and 2 mM glutamine. The HeLa, L(tk$^-$) and RAG cell lines were maintained in DMEM supplemented with 10% fetal calf serum (FCS), antibiotics, and 2 mM glutamine under standard conditions.

Recombinant human and mouse IFNγ were kindly provided by Roussel-Uclaf, and their specific activities were estimated by anti-viral assays as previously described (Bono et al. 1989). Human and mouse IFNγ were used at 500 units/ml or 250 units/ml, respectively.

Cell fusions

All the fusions were performed with 10^7 cells of each fusion partner using PEG 4000 (Merck), and following previously described protocols (Bono et al. 1991). The adherent hybrids generated by the fusion of the A20 B-LCL with the RAG cell line, which is adherent, and *HPRT*$^-$ (hypoxanthine phosphorybosyltransferase), were simply selected in DMEM plus HAT, since the A20 cells are *HPRT*$^+$ and non-adherent. The same selection medium was used to obtain the hybrids between the HeLa (adherent, *HPRT*$^-$) and Daudi (non-adherent, *HPRT*$^+$) cell lines. For the HeLa × A20 hybrids, we used neomycine-resistant A20 cells, resulting from the electroporation of A20 with the pSV2neo plasmid (Southern and Berg 1982). Two, 3, 4, and 10 days after fusions, three washes with fresh medium were performed to eliminate the non-adherent cells. The hybrids, maintained in DMEM plus 0.5 mg/ml Geneticine 418 (GIBCO), then began to adopt a globular morphology and become semi-adherent.

Antibodies

HLA-DR and Aα^d antigens were detected with monoclonal antibodies (mAbs) L112 (Bono et al. 1978) and MKD6 (Kappler et al. 1981), respectively. Human and murine MHC class I were detected with W6/32 (Barnstable et al. 1978) and 20.8.4 (Oi et al. 1978) mAbs, respectively.

Flow cytometric analyses

Sub-confluent cell cultures were treated for 72 h with IFNγ. Indirect immunofluorescence assays were done as described previously (Bono et al. 1989). The analysis was performed using a FACScan (Beckton Dickinson) with a Cell Quest program. Prior to the addition of the primary antibody, the A20 cell line and the HeLa × A20 hybrids were pre-incubated with 400 µl FCS containing 2 µg/ml unlabeled goat anti-mouse IgG (Biosys), to block mAb-unspecific binding to Fc receptors and surface immunoglobulins.

Luciferase constructs and assays

The pGL3-control, containing an SV40 promoter upstream of the luciferase gene, and the promoterless pGL3-basic plasmid constructs were purchased from Promega. The CIITA-1783-luc construct, which contains a 1783 base pair (bp) region upstream of the coding region of the human *CIITA* gene, is able to drive high luciferase expression in B-LCLs, and has been described elsewhere (Lennon et al. 1997). Transient transfections were performed by electroporation of the luciferase constructs using a Gene Pulser apparatus (Bio-Rad, Calif.). For the Daudi cell lines, 5.10^6 cells were resuspended in 500 µl of RPMI 1640 supplemented with 10% FCS and containing 20 µg of luciferase plasmid DNA mixed with 2 µg of the pSVβgal plasmid (Promega). Cells were electroporated with a 960 µF/300 V/0 Ω pulse. For the RAG, HeLa, A20 cell lines, and all the hybrids, 5.10^6 cells were resuspended in 200 µl of either RPMI 1640 or DMEM containing 10% FCS and 20 µg of luciferase construct DNA mixed with 2 µg

of the pSVβgal plasmid. A 960 μF/300 V/200 Ω pulse was then applied.

The following day, the transfectants were washed in fresh media, then, when necessary, treated for 16 h with either mouse or human IFNγ. The cell extracts were prepared 48 h after transfection and assayed in the Luciferase Assay System (Promega), according to the manufacturer's instructions. The same cellular extracts were assayed for β–galactosidase activity as described elsewhere (Sambrook et al. 1989). Luciferase activities, measured in relative light units per s (RLU.s^{-1}) with the Lumat luminometer (Berthold Instruments), were divided by the β–galactosidase activities (expressed as A_{420}) to correct for transfection efficiency. The luciferase activity of CIITA-1783-luc was calculated substracting the value obtained with pGL3-basic (promoterless) luciferase vector, and subsequently expressed as a percentage of the value obtained with the pGL3-control plasmid (containing a SV40 promoter). All the values correspond to an average of three independent experiments.

Stable transfections with the recombinant CIITA cDNA

pREP4 (InVitroGen, San Diego, Calif.) is an Epstein-Barr virus (EBV)-based vector with the hygromycin-resistance gene (HygroR). The pREP4/CIITA vector contains the 4.5 kilobase (kb) cDNA encoding the human CIITA (Steimle et al. 1993) driven by a Rous sarcoma virus long terminal repeat promoter. 5.10^6 HeLa × Daudi hybrid clones 2 and 3, or HeLa cells were resuspended in 200 μl of DMEM containing 10% FCS and 20 μg of pREP4 or pREP4/CIITA vectors. Cells were then electroporated with a 960 μF/300 V/200 Ω pulse. Forty-eight h after the transfection, hygromycin (0.3 mg/ml) was introduced in the medium, and the pool of hygromycin-resistant cells was analyzed after a 3-week selection.

Reverse transcription-polymerase chain reaction

Total RNA was isolated from subconfluent cultures using the guanidinium thiocyanate method (Chirgwin et al. 1979). One microgram of total RNA was incubated for 1 h at 42 °C in the presence of the anti-sense oligonucleotides, 2 units AMV reverse transcriptase (Promega), 15 units RNAsin, 1 mM dNTP, 2.5 mM $MgCl_2$, in the *Taq* buffer from the Ampli*Taq* kit (Perkin Elmer Cetus), in a 20 μl final volume. The polymerase chain reactioon (PCR) was then performed on the resulting cDNA in a 100 μl volume (200 μM dNTP; 2.5 mM $MgCl_2$) after introduction of the sense oligonucleotides and 5 units *Taq* polymerase. All the PCRs were performed on a DNA Thermal Cycler (Perkin Elmer Cetus) with the following cycles: 95 °C, 5 min, one cycle; 95 °C, 1 min, 55 °C, 1 min, 72 °C, 2 min, 30 cycles; 72 °C, 10 min, one cycle. Human *CIITA* RNA was amplified using the H.C primer set (100 ng) (H.C.sense: 5′-CGCCCTATTTGAGCTGTC-3'; H.C.anti-sense: 5′-GGTCAATGCTAGGTACTG-3'; the amplified 538 bp-fragment corresponds to nt. 1798–2336 of the human cDNA (Steimle et al. 1993)). The mouse *CIITA* RNA was amplified with a set of primers (100 ng) (M3′-sense: 5′-CTGGATCGTCTCGTGCAG-3'; M3′-anti-sense: 5′-CCATGTCCGGAAGTACTT-3'; the amplified 424 bp DNA fragment corresponds to nt. 2728–3152 of the mouse cDNA (Sims et al. 1997). In order to control the amplification efficiency, 15 ng of non-species-specific β–actin primers (Chang et al. 1994) (β-Act-sense: 5′-CACCCTGTGCTGCTCACCGAGGCC-3'; β-Act-anti-sense: 5′-CCACACAGAGTACTTGCGCTCAGG-3′) were introduced in each of the RT-PCRs.

Northern blot analysis

Total RNA was isolated as described for reverse transcription (RT)-PCR. RNA samples (15 μg per lane) were loaded on agarose gels containing 2.2 M formaldehyde. The gels were transferred onto Nylon N membranes (Amersham) and the filters were hybridized overnight with probes labeled by the random-priming technique using the Multiprime Labelling Kit from Amersham.

DNA probes

The *A*α^d, *HLA–DQB, HLA-DMB,* mouse–*Ii*, and β–*actin* probes have been previously described (Lennon et al. 1996). The *HLA-DMB* probe cross-hybridizes with the *HLA-DMA* and mouse *H-2M* mRNAs. The mouse *Ii* probe cross-hybridizes with its human homologue.

Results

Suppression of MHC class II expression in intraspecies hybrids

We first generated intraspecies hybrids where most chromosomes are maintained (Gourdeau and Fournier 1990), and biases linked to the presence of species-specific factors are avoided. Human intraspecies hybrids were generated by the fusion of MHC class II^- HeLa epithelial cells with MHC class II^+ Daudi B-LCLs (H.D. hybrids). Mouse intraspecies hybrids were obtained by the fusion of MHC class II^- RAG epithelial cells with MHC class II^+ A20 B-LCLs (R.A. hybrids). We have previously shown that the RAG cell line is defective for MHC class II expression, even after an IFNγ treatment, and that re-expression was observed in somatic cell hybrids obtained from the fusion of RAG with various human cell lines (Bono et al. 1991). The H.D. and R.A. hybrids obtained in both cases are adherent cells. Twenty clones per fusion were analyzed by indirect immunofluorescence for the expression of cell-surface MHC class II antigens. The data obtained with two representative clones per hybrid type (H.D.2, H.D.3, R.A.11, and R.A.12) are displayed in Fig. 1. For all the analyzed clones, we did not observe any constitutive expression of MHC class II glycoproteins at the cell surface. However, when the somatic cell hybrids were treated with IFNγ, MHC class II expression was induced, demonstrating that the absence of expression is due to an active phenomenon, and is not caused by a simple loss of the MHC class II-encoding chromosome in the hybrids. In addition, in all the analyzed hybrid clones, MHC class I antigens were expressed constitutively at levels similar to that of the epithelial parental cell line, and their expression was stimulated when the cells were treated by IFNγ (data not shown). These results indicate that the suppression mechanism is MHC class II-specific, and does not affect the expression of MHC class I genes.

In order to assess whether the MHC class II silencing was not limited to the four parental cell lines described above, the same analysis was performed with mouse hybrids of the MHC class II-negative L(tk^-) epithelial cell line and A20 B-LCL, and with human hybrids of HeLa cells and the MHC class II-positive mon-

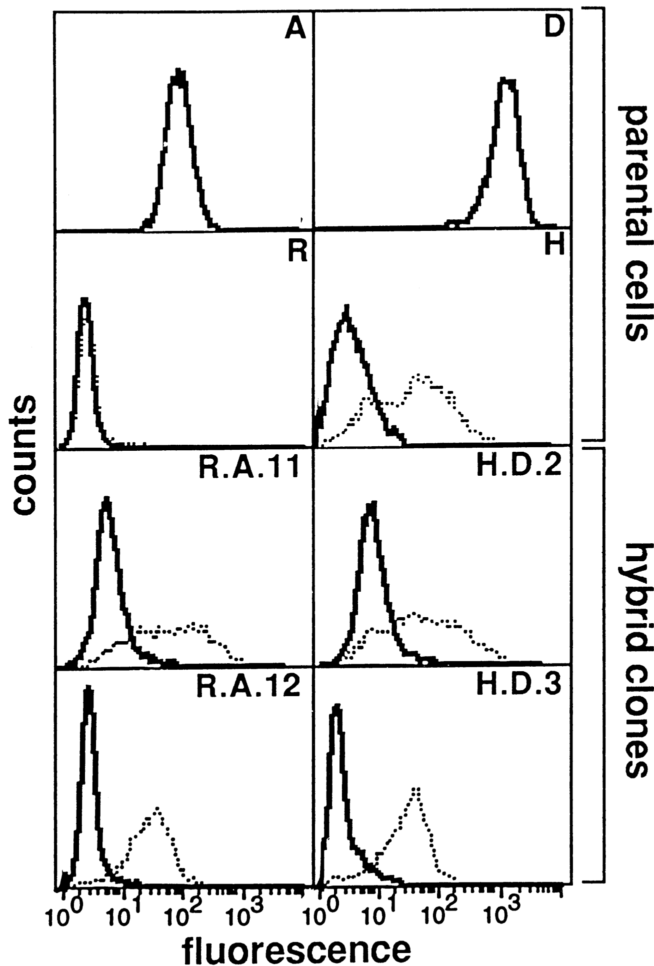

Fig. 1 Flow cytometric analysis of cell surface MHC class II expression in parental cells and hybrid clones. Daudi (D), Hela (H), Hela × Daudi clone 2 (H.D.2), and clone 3 (H.D.3) were untreated (*solid lines*) or treated (*dotted lines*) with human-IFNγ for 72 h, and indirect immunofluorescence was performed using a DR-specific antibody. A20 (A), RAG (R), RAG × A20 clone 11 (R.A.11) and clone 12 (R.A 12) were analyzed under similar conditions except that mouse-IFNγ was used and indirect immunofluorescence was made with an I-A^d-specific antibody

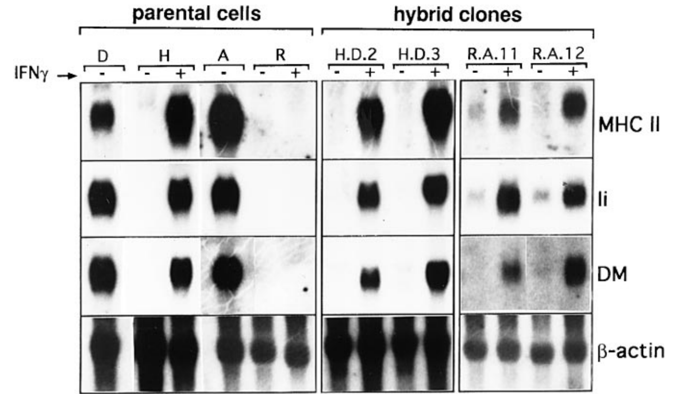

Fig. 2 Northern blot analysis of MHC class II, Ii, and HLA-DM/H–2M expression in parental cells and hybrids clones. Daudi (D), Hela (H), Hela × Daudi clone 2 (H.D.2), and clone 3 (H.D.3) were untreated (–) or treated (+) with human IFNγ for 48 h prior to extraction. The membranes were successively hybridized with the *DQB*, or mouse *Ii*, or *HLA-DMB* probes. A20 (A), RAG (R), RAG × A20 clone 11 (R.A.11), and clone 12 (R.A 12) were analyzed similarly, except that mouse-IFNγ was used and the membranes were hybridized with the $A\alpha^d$, or mouse *Ii*, or *HLA-DMB* probes. An actin probe was used to control the amount of RNA per lane

ocyte-macrophage KG1 cell line. Again, we observed a MHC class II-specific suppression which was relieved after an IFNγ incubation (data not shown).

In order to determine whether the suppression phenomenon was affecting the MHC class II transcript level and whether it was also modifying transcription of the *Ii* and *HLA-DM/H2M* genes, northern blot analyses were next performed. In human H.D.2 and H.D.3 hybrid cell clones, MHC class II transcripts were only detected in the IFNγ-treated cells (Fig. 2). Similar data were obtained for the *Ii* and *HLA-DM* genes. In the mouse R.A.11 and R.A.12 hybrid clones, MHC class II, *H-2M,* and *Ii* mRNAs were induced by IFNγ as well, but a low basal level of transcripts was detected in these hybrids in the absence of the cytokine. Interestingly, in the mouse L(tk-) × A20 hybrids, the same expression pattern was obtained (data not shown). Therefore, these results indicate that the silencing observed in human or mouse intraspecific hybrids coordinately affects the expression of genes whose products are involved in antigen presentation by the MHC class II molecules. The suppression process alters the transcription of these genes, and can be bypassed by an IFNγ treatment, which then allows their mRNA accumulation.

Analysis of MHC class II expression in inter-species hybrids

Our next objective was to verify whether the suppression was an active phenomenon, and whether it was linked to the presence of chromosome(s) from one of the fusion partners. It was then interesting to use the human chromosome segregation which usually occurs in mouse × human inter-species hybrids (Gourdeau and Fournier 1990). In a previous report, we have demonstrated that in more than 30 hybrid clones generated by the fusion of a mouse epithelial-like cell (RAG) with various human B-LCLs, MHC class II silencing was consistently observed, independently of the variable human chromosome content of the hybrids (Bono et al. 1991). In good agreement with the data presented above, a coordinate silencing of MHC class II, *Ii*, and *HLA-DM/H-2M* gene transcription was obtained in the hybrid clones analyzed elsewhere by northern blot (Lennon et al. 1996). We then hypothesized that the MHC class II suppression process might be linked to the presence of chromosomes provided by the epithelial fusion partner, and that the silencing should then be relieved by the segregation of these chromosomes.

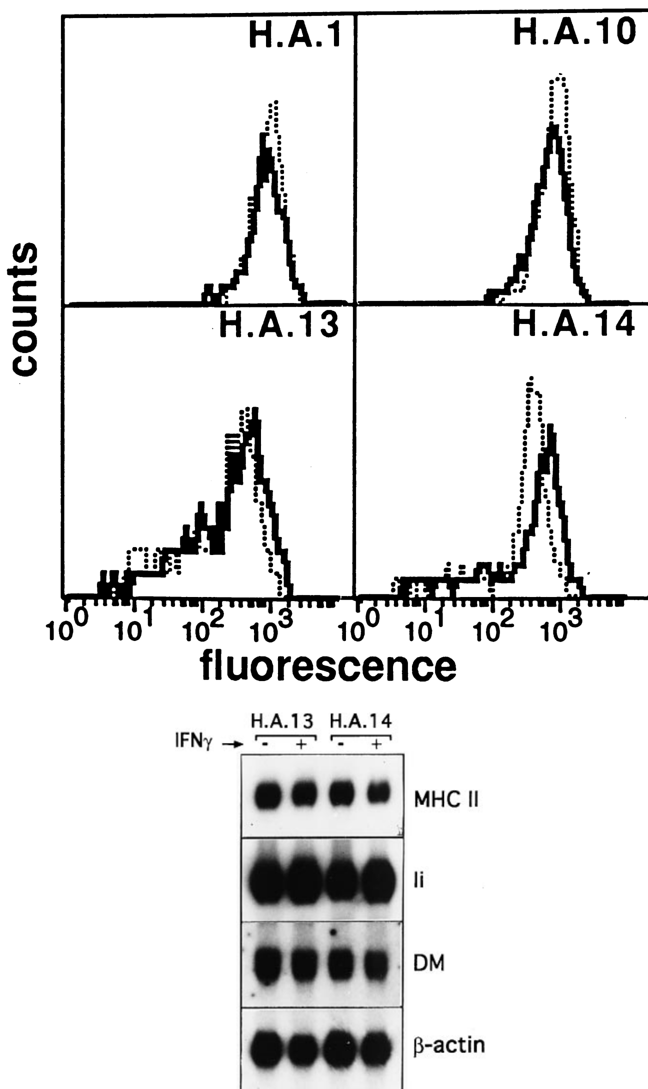

Fig. 3 **A** Flow cytometric analysis of surface mouse MHC class II expression in Hela × A20 clone 1 (H.A.1), clone 10 (H.A.10), clone 13 (H.A.13), and clone 14 (H.A.14). Cells were untreated (*solid lines*), or treated (*dotted lines*) with mouse IFNγ for 72 h. Indirect immunofluorescence was performed using a I-A^d–specific antibody. **B** Northern blot analysis of Aα^d, *Ii*, and *HLA-DM/H-2M* expression in Hela × A20 clone 13 (H.A.13) and clone 14 (H.A.14). Cells were untreated (–), or treated (+) with mouse IFNγ for 48 h before RNA extraction. See the control parental cell lines in Fig. 1. An actin probe was used to control the amount of RNA per lane

Human epithelial cells × mouse B-LCLs hybrids were therefore generated through the fusion of the human epithelial HeLa cells (MHC class II-negative) and the mouse A20 B-LCL (MHC class II-positive). During the selection procedure, we noticed that the resulting hybrid clones progressively lost an adherent epithelial-like phenotype to become semi-adherent cells and sometimes suspended cells. A mixture of these two latter morphologies was maintained after selection. These observations were in good agreement with the expected loss of human chromosomes which were provided by the epithelial HeLa cell line. The hybrid nature of these cells was assessed by the co-expression of both mouse and human MHC class I cell-surface antigens in all the clones analyzed (data not shown).

We then examined by indirect immuno fluorescence the expression pattern of cell-surface mouse MHC class II antigen expression in ten independent HeLa × A20 (H.A.) hybrid clones. For seven clones, a constitutive expression of Aαd antigens was detected (see, Fig. 3A, where clones H.A.1 and H.A.10 are depicted as an example). For three of the ten clones (see Fig. 3A, where clones H.A.13 and H.A.14 are presented), a heterogeneous pattern was observed: depending on the hybrid analyzed, 10–30% of the cells were MHC class II-negative, and the remainder of the cells were presenting constitutive MHC class II expression. Quite interestingly, these three clones were analyzed in a similar manner 2 weeks later, and it was then observed that they had become homogeneous for the constitutive expression of mouse MHC class II cell-surface antigens (data not shown). These results were also confirmed with the analysis of the expression of HLA-DR which was detected at a low constitutive level in five of the ten hybrid clones (data not shown). Consequently, both the lymphocyte-like morphology of these hybrids, and the progressive relieving of the MHC class II silencing during cell culture, suggest that the suppression phenomenon is linked to the presence of the segregant chromosomes which were originally provided by the human epithelial cell line.

Treatment of the H.A. hybrid clones with mouse IFNγ did not induce the expression of mouse MHC class II antigens, except in one clone which displayed a weak induction. These hybrids respond however to IFNγ, as the mouse MHC class I antigen expression is stimulated by the cytokine (data not shown). These data did not surprise us, as in all the EBV-established B-LCLs and lymphomas we have analyzed to date, we have never detected any MHC class II stimulation by this cytokine (unpublished data).

Northern blot analysis confirmed the constitutive expression of mouse MHC class II, *Ii,* and *HLA-DM/H-2M* mRNAs in the HeLa × A20 hybrid clones (see Fig. 3B, clones H.A.13 and H.A.14).

Analysis of CIITA expression in the hybrids

The data presented above establish a coordinate regulation of MHC class II, *Ii*, and *HLA-DM/H-2M* gene expression in either the MHC class II-negative (intraspecies R.A and H.D.) or the MHC class II-positive (interspecies H.A.) hybrids. As it has been demonstrated that CIITA is required for the transcription of all these genes (Chang and Flavell 1995), it became interesting to check whether *CIITA* mRNA expression was affected in a similar manner in the hybrids. In the MHC class II-negative human H.D. and mouse R.A. hybrids, we observed, by RT-PCR, a weak constitutive expres-

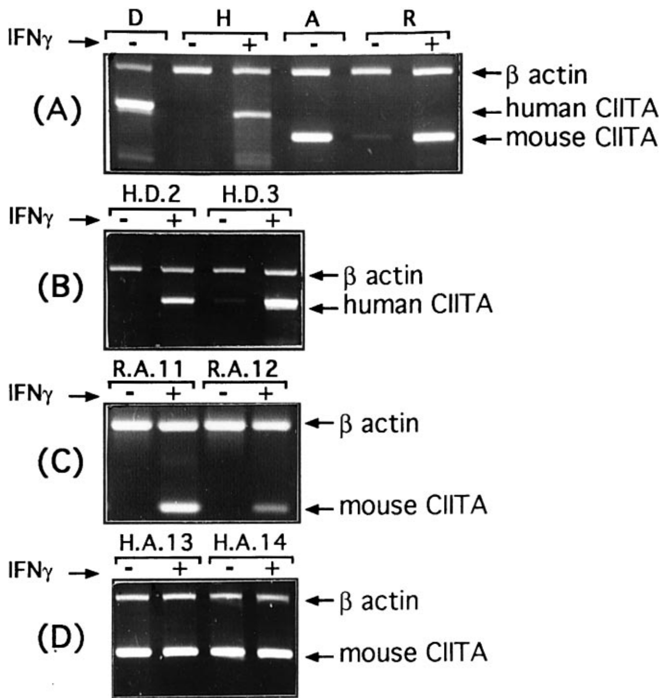

Fig. 4A–D RT-PCR analysis performed with primers specific for either human or mouse *CIITA* mRNA. Actin primers were introduced in the PCR mix to control RNA amount and amplification efficiency. Total mRNA of the following cells was used: **A** Daudi (D), Hela (H), A20 (A), RAG (R), untreated (–) or treated (+) with human or mouse-IFNγ. **B** Hela × Daudi clone 2 (H.D.2), clone 3 (H.D.3), untreated (–) or treated (+) with human-IFNγ. **C** RAG × A20 clone 11 (R.A.11) and clone 12 (R.A 12), untreated (–) or treated (+) with mouse-IFNγ. **D** Hela × A20 clone 13 (H.A.13) and clone 14 (H.A.1), untreated (–) or treated (+) with mouse-IFNγ

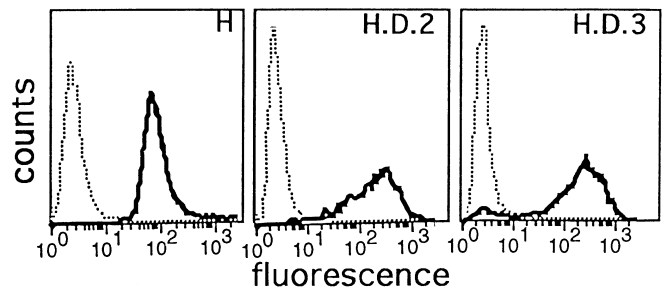

Fig. 5 Flow cytometric analysis of cell-surface HLA-DR antigens in HeLa cell line (H) and the H.D.2 and H.D.3 hybrid clones stably transfected with either pREP4 (*dotted lines*) or pRE4/CIITA (*solid lines*)

sion of the mouse *CIITA* mRNA with a level similar to that of the epithelial parental cell lines (Fig. 4). IFNγ treatment increased *CIITA* mRNA accumulation in these cells. In contrast, in the MHC class II-expressing H.A. hybrids, mouse *CIITA* mRNA is constitutively expressed at a high level comparable to that of the parental A20 B-LCL. Therefore, the expression pattern of mouse *CIITA* is similar to that of the MHC class II genes. It is interesting to note that in most H.A. hybrids, human *CIITA* mRNA expression was not detected, which is probably explained by the segregation of human chromosome 16-encoding CIITA (Steimle et al. 1994).

These experiments therefore suggested that the MHC class II suppression phenomenon observed in the intraspecies hybrids results from a strong decrease in the amount of the CIITA transcript. We next addressed the question of whether the artificially-provoked expression of *CIITA* mRNA in the MHC class II-negative hybrids would restore MHC class II expression. We then stably transfected the cDNA encoding the human CIITA placed under the control of a heterologous promoter in two silenced human intraspecies hybrid clones, namely H.D.2 and H.D.3, in parallel with the human epithelial fusion partner, HeLa. As displayed in Fig. 5, mock-transfection did not alter the MHC class II-negative phenotype of either cell line. In contrast, the over-expression of CIITA in both H.D. hybrid clones and in the HeLa cell line restores expression of the HLA-DR cell-surface antigens. These data further indicate that the de novo expression of exogeneous CIITA in the hybrids can bypass the MHC class II suppression process.

CIITA B-cell-specific promoter activity in the hybrids

The highly reduced amount of constitutively expressed *CIITA* mRNA in the silenced hybrids can be mediated either by the repression in a transcriptional initiation, or by an increased instability of the transcript. We therefore analyzed the activity of a *CIITA* promoter that we had previously isolated (Lennon et al. 1997). The CIITA-1783-luc vector contains a 5' flanking region of *CIITA* gene which was cloned into a luciferase reporter gene construct. This vector is able to drive strong constitutive luciferase expression when transfected into human and mouse B-LCL, but is poorly active when introduced into macrophage or fibroblast cell lines where it is not responsive to IFNγ.

We then examined the activity of this *CIITA* B-cell-specific promoter by transfecting the CIITA-1783-luc construct in two clones of the MHC class II-negative mouse R.A. and human H.D. hybrids, and in three clones of the MHC class II-positive H.A. hybrids. The results (Fig. 6) show that the luciferase expression driven by the CIITA-1783-luc construct in the H.D. and R.A. hybrid clones is similar to that obtained with the epithelial parental cell line. In contrast, in the H.A. hybrids (only two of the three clones analyzed are displayed in Fig. 6, but the results obtained with the third clone are equivalent), the luciferase expression level is not reduced when compared with the A20 B-LCL, and is even as high as that obtained with the Daudi B-LCL. As expected from our previous data (Lennon et al. 1997), the luciferase activity was not induced by IFNγ in any cell hybrids (data not shown). The expression

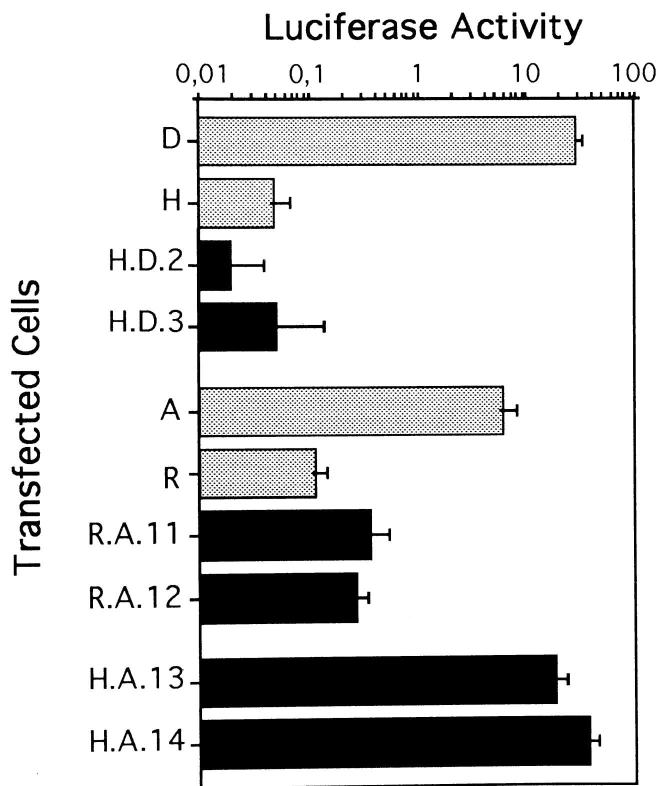

Fig. 6 Luciferase assay with the CIITA-1783-luc construct. Luciferase intensity values were standardized relative to the transfection efficiency of the co-introduced pSVβgal plasmid, assayed by a quantitation of the β-galactosidase activity. All the assays were performed in parallel with the promoterless vector, pGL3-basic, in order to control its background luciferase expression, and with the pGL3-control plasmid, in which the luciferase gene is under the control of an SV40 promoter. The luciferase activity of CIITA-1783-luc was calculated substracting the value (considered as 100%) obtained with pGL3-basic vector, and subsequently expressed as a percentage of that obtained with the pGL3-control plasmid. The assays were performed in the following cells: Daudi (D), Hela (H), Hela × Daudi clone 2 (H.D.2) and clone 3 (H.D.3), A20 (A), RAG (R), RAG × A20 clone 11 (R.A.11), and clone 12 (R.A 12), Hela × A20 clone 13 (H.A.13) and clone 14 (H.A.14)

pattern of *CIITA* observed in these hybrids is therefore the reflection of the transcription level driven by the *CIITA* B-cell-specific promoter. These data infer that the silencer suppresses expression of the *CIITA* mRNA transcribed from its B-cell-specific promoter.

Discussion

The analysis of cell hybrids reported here aimed towards the understanding of MHC class II tissue-specific pattern of expression. We first established MHC class II silencing was observed in either human or mouse intraspecies hybrids between MHC class II-positive B-LCLs and MHC class II-negative epithelial cell lines. These data are in good agreement with a publication by another group (Stuart et al. 1989) on mouse intraspecies B-LCL × epithelial cell hybrids. As MHC class II suppression in the intraspecies hybrids is relieved in the presence of IFNγ, the phenotype of these hybrids is therefore similar to that of an epithelial cell line, suggesting that the silencing was mediated by the epithelial fusion partner.

In order to assess this hypothesis, we next studied human × mouse interspecies hybrids, in which human chromosome segregation is usually observed (Gourdeau and Fournier 1990). Hybrid clones generated by the fusion of a human MHC class II-negative epithelial cell line with a mouse MHC class II-positive B-LCL adopted a semi-adherent morphology during the three-week selection. This phenomenon is explained by the segregation of the chromosomes provided by the adherent epithelial fusion partner. Most of the hybrid clones were expressing MHC class II, thereby resembling the parental B-LCL. Interestingly, three other clones were displaying a heterogeneous pattern of expression which was lost after two additional weeks of cell culture. In these hybrids, the segregation phenomenon was so rapid that we were not able to isolate hybrids with a homogeneous MHC class II-negative phenotype, which would have been useful for a correlation between the karyotype and the phenotype of the cells. These data are in accordance with our hypothesis concerning a suppression effect mediated by the epithelial cell, as the loss of human chromosomes drives the hybrids towards a MHC class II-expressing phenotype. In addition, our results show that the suppression process is reversible.

For all the hybrids presented here and in a previous publication (Lennon et al. 1996), we established that a coordinate pattern of expression of MHC class II, *Ii,* and *HLA-DM/H-2M* genes was consistently observed. In addition, a similar pattern of expression of *CIITA* mRNA was observed in the cell hybrids. Our results demonstrate therefore that the silencing affects accumulation of the CIITA transcript, suggesting that MHC class II suppression results from a lack of CIITA. These data are in agreement with those recently obtained in hybrids between B-LCLs and plasmocytes (Sartoris et al. 1996). However we can not rule out the possibility that different silencing mechanisms operate in epithelial cells and plasmocytes.

We next analyzed the activity of the B-cell-specific promoter of human *CIITA* in the hybrid cells. In the silenced hybrids the expression level of the *CIITA* B-cell-specific promoter was similar to that obtained with the epithelial parental cell lines. Conversely, in the MHC class II-positive H.A. hybrids, the level of expression of the luciferase construct was high. The promoter activity even reached that observed in the Daudi cell line, which is four times higher than that obtained in the A20 cells. As the *CIITA* B-cell-specific promoter tested here is human, it is likely that the remaining human chromosomes in the hybrids allow a better transcription than the mouse transcription factors.

These data therefore show that the B-cell-specific *CIITA* promoter is the actual site of silencing in MHC class II-negative somatic cell hybrids. A deletion analysis of this promoter might lead to constructs that could confer constitutive expression of the promoter in epithelial cell lines and thereby allow identification of the promoter region affected by the silencing process. This suppressor might exert its function directly, by binding to the *CIITA* promoter, and thereby repress transcription initiation. The silencing mechanism could also alter the activity or the binding of a positive transcription factor necessary for the transcription of CIITA. Alternatively, more complex schemes might play a role, with the activation or repression of intermediate genes. Methylation may also be involved, as in the hepatic differentiation system it has been shown that extinction of the albumin gene is related to its methylation state (Sellem et al. 1985; Sperling et al. 1984). Whether activity of the B-cell-specific *CIITA* promoter might be modulated by methylation remains to be investigated.

The next important point involves the mechanism relieving the silencing when the MHC class II negative hybrids are treated by IFNγ. We have previously established the existence of a B cell-specific promoter in the *CIITA* gene (Lennon et al. 1997). A recent publication (Muhlethaler-Mottet et al. 1997) confirmed that multiple promoters were controlling the expression of *CIITA* in different tissues, and showed that an IFNγ-responsive promoter was located 2 kb downstream of the B-cell-specific promoter. On the basis of the data presented here, one can therefore hypothesize that, in epithelial cell lines, the weak expression of the *CIITA* B-cell promoter is due to an active suppression of transcription. When the cells are treated with IFNγ, the transcriptional machinery is directed towards an IFNγ-responsive promoter.

Therefore, CIITA appears to be the central element in MHC class II expression, as several different pathways involved in MHC class II regulation of expression converge towards the *CIITA* gene (Mach et al. 1996). This regulation mode appears to be advantageous because it ensures that all MHC class II genes, in addition to *Ii* and *HLA-DM,* will be co-expressed whenever required. As a dyscoordinate over-expression of some of these genes might lead to the presentation of autoantigens (Pieters 1997), and therefore to autoimmune reactions, the silencing of *CIITA* in non-professional APCs can be interpreted as a protection strategy.

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