MHC class II-deficient tumor cell lines with a defective expression of the class II transactivator

Rodrigo Naves1, Ana Maria Lennon2, Giovanna Barbieri3,4, Lilian Reyes1, Gisella Puga1, Laura Salas2, Virginie Deffrennes3, Mario Rosemblatt1, Marc Fellous2, Dominique Charron3, Catherine Alcaide-Loridan3 and Maria Rosa Bono1

1Departamento de Biologia, Facultad de Ciencias, Universidad de Chile, and Millennium Institute for Fundamental and Applied Biology, Casilla 653, Santiago, Chile
2Unité d’Immunogénétique Humaine, INSERM U276, Institut Pasteur, 25 rue du Dr Roux, 75724 Paris Cedex 15, France
3INSERM U396, Centre de Recherches Biomédicales des Cordeliers, 15 rue de l’Ecole de Médecine, 75006 Paris, France
4Permanent address: Istituto di Biologia dello Sviluppo, CNR 152 via Ugo La Malfa, 90146 Palermo, Italy

Keywords: CIITA, MHC class II, tumor cell line, transcription

Abstract

MHC class II expression defects have been evidenced in several human tumor cell lines originating from lung cancers or retinoblastoma. Accordingly, the mouse adenocarcinoma and fibrosarcoma cell lines, RAG and L(tk±), do not express I-A and I-E molecules even when treated with IFN-γ. Here we show that fusion of both cell lines restores the inducible expression of MHC class II, thereby demonstrating that they present different and recessive alterations outside the MHC class II locus. CIITA, the MHC class II transactivator, controls the tissue-specific expression of MHC class II genes and creates the architecture of the transcriptional complex that binds to the MHC class II gene promoters. In L(tk±) cells, C2ta transcripts, expressed from the gene encoding CIITA, were indeed detected in severely limited amounts, with a defect in C2ta transcription initiation. In agreement we show here that the L(tk±) cell line does not express the CIITA protein. In contrast, in the RAG cell line, C2ta transcripts were expressed at normal levels, from the proper initiation site. The nucleotide sequencing of the CIITA cDNA from RAG did not reveal any mutation. However, the CIITA protein was not detected. These data evidence a new type of defect in a MHC class II-defective tumor cell line, as we show here that the alteration in the RAG cells occurs downstream of C2ta transcription. The RAG mutation might therefore reside in the C2ta transcript nuclear export or translation, or in the stability of the CIITA protein.

Introduction

MHC class II molecules play a central role in the presentation of antigens to CD4+ T lymphocytes during the initiation of the immune response (1). Constitutive expression of MHC class II molecules is restricted to professional antigen-presenting cells (APC) including B lymphocytes, dendritic cells and activated macrophages. Non-professional APC like endothelial cells, astrocytes, epithelial cells or fibroblasts can be induced to express MHC class II by IFN-γ (2). MHC class II gene expression is mainly regulated at the transcriptional level by four conserved cis-acting promoter elements termed W, X1, X2 and Y boxes (2,3).

Genetic complementation analysis carried out with cell lines derived from patients affected by MHC class II deficiency (4) defined four complementation groups (groups A–D) (5). Cell lines from complementation groups B, C and D display mutations in RFXANK (6), RFX5 (7) and RFXAP (8) respectiv-
Defective CIITA expression in tumor cells

Methods

Cell lines and IFN

RAG (a mouse adenocarcinoma cell line), L(tk-') (a mouse fibrosarcoma cell line), WISH (a human amniotic cell line), RAW264.7 (a mouse monocyte-macrophage cell line), J777.4 (a mouse pre-monocyte cell line) and A20 (a mouse B cell lymphoma) were obtained from ATCC (Rockville, MD). The LM and GES human B cell lines were immortalized with Epstein–Barr virus. The RAW264.7 and WISH cell lines express MHC class II molecules after IFN-γ induction. The LM and GES cell lines constitutively express MHC class II molecules. The R.GES.2.1 cell hybrid originates from the fusion of the RAG and GES cell lines, and contains different human chromosomes including chromosome 16 (25). The RR2.16 cell line is a radiation somatic cell hybrid prepared from the R.2417.2 somatic cell hybrid (25). The resulting RR2.16 hybrid presents the genetic background of the RAG cell line and contains fragments of human chromosome 16 (our unpublished data). The RAG, J777.4, R.GES.2.1, RR2.16 and L(tk-) cell lines were grown in DMEM supplemented with 10% FCS, antibiotics and 2 mM glutamine. The A20, RAW264.7, WISH, GES and LM cells were maintained in RPMI 1640 supplemented with 10% FCS, antibiotics and 2 mM glutamine under standard conditions. The specific activity of the mouse IFN-γ (kindly provided by Roussel-Uclaf, Romainville, France) was estimated by antiviral assay as previously described (26) and it was used at 150 or 250 U/ml as indicated in the figure legends.

Flow cytometric analyses

Subconfluent cell cultures were treated for 72 h with IFN-γ. Indirect immunofluorescence assays were performed using the following primary mAb: 20.8.4 (anti-H-2d) (27), MKD6 (anti-I-Ak) (28), 11.4.1 (anti-H-2k; ATCC TIB 95) and H39.64.5 (anti-I-Ak) (28). Cells were next labeled with FITC-conjugated sheep anti-mouse Ig from Biosys (Compiegne, France). Flow cytometric analysis was performed on a FACScan (Becton Dickinson, Mountain View, CA) using the Lysys II program (Becton Dickinson).

Cell fusions

Somatic cell hybrids were prepared by the fusion of 10^7 cells of each fusion partner using PEG 4000 (Merck, Darmstadt, Germany) following previously described protocols (29). Adherent hybrids generated by the fusion of the L(tk-') cell line (thymidine kinase-deficient) with GES (L.GES) or LM (L.LM) cells were selected in DMEM plus HAT while non-adherent GES and LM cells were eliminated by washing. Hybrids obtained by the fusion of L(tk-') and WISH cell lines (L.W) were selected on DMEM containing HAT plus 1.0 mg/ml G418 (Geneticin; Gibco, Rockville, MD) since the adherent WISH cells were made G418-resistant by transfection with a pSV2neo plasmid (30) by calcium phosphate precipitation (31) prior to the fusion

PCR

All PCR reactions were performed on a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) with the following cycles: one cycle of 4 min at 95°C, 30 cycles of 1 min at 95°C, 1 min at 55–65°C, 2 min at 72°C and finally one cycle of 10 min at 72°C. For each PCR reaction, the annealing temperature used was near to the melting temperature of each primers. Human genes were amplified with the following human-specific sense (S) and antisense (AS) primers sets: CIITA: H.C-S (5'-CGCCCTATTGGTCTGTC-3') and H.C-AS (5'-GGTCAAATGTCAGTACTG-3'); RFX-5: HRFX5-S (5'-CCAATCCAGTCTCCAC-3') and HRFX5-AS (5'-CCACCTATGCGCTACCT-3'); RFXAP: HRFXAP-S (5'-GGTCTCATACAGGGTCAAT-3') and HRFXAP-AS (5'-TAGTTAATCCGTCAGTTT-3'); protamine I: PRM1-S (5'-GCTTGGCACACTAGC-3').
Defective CIITA expression in tumor cells

Table 1. Sequence and position of the different primers used for the amplification and sequencing of the CIITA cDNA

<table>
<thead>
<tr>
<th>Primer designation</th>
<th>Primer sequence</th>
<th>Position (nucleotides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM37S</td>
<td>5'-CTCAGAAACCGACGAGCAGG-3'</td>
<td>53-73</td>
</tr>
<tr>
<td>CM10S</td>
<td>5'-GGGAGGCTCCGGAACGCAGG-3'</td>
<td>24-43</td>
</tr>
<tr>
<td>CM11S</td>
<td>5'-CGACCACCTCTAATCTTACCC-3'</td>
<td>218-238</td>
</tr>
<tr>
<td>CM12AS</td>
<td>5'-CTGAGGCGTCTGCTGAGG-3'</td>
<td>758-738</td>
</tr>
<tr>
<td>CM43S</td>
<td>5'-CTCCCTTCAAGGACGCTCAG-3'</td>
<td>739-758</td>
</tr>
<tr>
<td>CM2S</td>
<td>5'-TCAGAAGCGCTCTAGCT-3'</td>
<td>745-761</td>
</tr>
<tr>
<td>CM2AS</td>
<td>5'-TCTGAGGATGACGAGCAG-3'</td>
<td>1433-1417</td>
</tr>
<tr>
<td>CM15.1S</td>
<td>5'-GAGCGATTCCAACACTCCTC-3'</td>
<td>952-971</td>
</tr>
<tr>
<td>CM32AS</td>
<td>5'-CTCAGAATCCTGAGAACCTG-3'</td>
<td>1110-1140</td>
</tr>
<tr>
<td>CM13S</td>
<td>5'-GCCAGACCTGTTCGCTCCTA-3'</td>
<td>1407-1426</td>
</tr>
<tr>
<td>CM14AS</td>
<td>5'-CGGAGGACTGTTCTGAGCT-3'</td>
<td>1900-1881</td>
</tr>
<tr>
<td>CM22S</td>
<td>5'-GAGGGCTGACACTGTCCTCAGC-3'</td>
<td>1553-1574</td>
</tr>
<tr>
<td>CM22AS</td>
<td>5'-GAGGCTCCTGCTGCTCAGG-3'</td>
<td>2050-2028</td>
</tr>
<tr>
<td>CM23S</td>
<td>5'-CATCAGGAGGAGCTGCAAACCTG-3'</td>
<td>1978-2000</td>
</tr>
<tr>
<td>CM23AS</td>
<td>5'-CTCCTGCTGACAGGAGACTC-3'</td>
<td>2647-2626</td>
</tr>
<tr>
<td>CM24S</td>
<td>5'-GCTAGCCACAGGGTGTACGCGA-3'</td>
<td>1110-1131</td>
</tr>
<tr>
<td>CM24AS</td>
<td>5'-CATGACTCTCCGCTGAGAACATC-3'</td>
<td>1551-1530</td>
</tr>
<tr>
<td>CM5S</td>
<td>5'-TGCGCTGACCCCATCCTGGC-3'</td>
<td>2535-2554</td>
</tr>
<tr>
<td>CM5AS</td>
<td>5'-CGGAGCCGCTCTGACCTGTC-3'</td>
<td>3200-3180</td>
</tr>
<tr>
<td>CM6S</td>
<td>5'-GGAGCCGAGAAAGCTGGCACA-3'</td>
<td>3115-3134</td>
</tr>
<tr>
<td>CM6AS</td>
<td>5'-CCCTTAGCTGCTGACAGGCC-3'</td>
<td>3786-3766</td>
</tr>
<tr>
<td>CM15S</td>
<td>5'-GGGCGCTGACCTGGTGTTTC-3'</td>
<td>3607-3626</td>
</tr>
<tr>
<td>CM16AS</td>
<td>5'-CCAGGACCAATCGTCCGTC-3'</td>
<td>4280-4260</td>
</tr>
<tr>
<td>CM27S</td>
<td>5'-ACACTGCTCTGATGACCGCCGCAGCTGCCGACAGCTGCC-3'</td>
<td>4450-4472</td>
</tr>
<tr>
<td>CM27AS</td>
<td>5'-CCCTGAGACAGCAGTCTTCTAC-3'</td>
<td>4989-4968</td>
</tr>
<tr>
<td>CM30S</td>
<td>5'-ATCTCTAGATGTCAGGAGGCCAG-3'</td>
<td>3803-3825</td>
</tr>
<tr>
<td>CM30AS</td>
<td>5'-GCACACCGGCAGATCGACAGTGT-3'</td>
<td>4472-4450</td>
</tr>
</tbody>
</table>

Numbering follows the sequence reported in (52) except for the primers noted with an asterisk, where the numbering is from (18).

GAGCTATTAG-3' and PRM1-AS (5'-CCCTTGCATCTCCTCCCTCAG3'); adenine phosphoribosyl transferase: APRT-S (5'-GGTGCTGACACTGTCCTGAC-3') and APRT-AS (5'-TGTCT CAACCTCTCTGAGCTCCCAA-3').

RT-PCR

Total RNA was isolated from subconfluent cultures using the guanidinium thiocyanate method (32). One microgram of total RNA was incubated for 1 h at 42°C in the presence of 200 ng anti-sense oligonucleotides, 2 U AMV reverse transcriptase (Promega, Madison, WI), 15 U RNAsin, 1 mM dNTP and 2.5 mM MgCl₂, in the Taq buffer from the AmpliTaq kit (Perkin-Elmer Cetus), in 10 μl final volume. PCR reactions were performed on the resulting cDNAs in a 10 μl final volume (200 μM dNTP; 2.5 mM MgCl₂) after addition of the sense primer (5′-CCCTGACGAGCTCTGACAGTACC-3′) and antisense primers described in Table 1. Amplification efficiency was assessed with non species-specific β-actin primers (15 ng) (β-Act-S: 5′-CACCCTGTGGCTGTCACCGAGGCC-3′; β-Act-AS: 5′-CCACACAGAAGCTTGGCCTGAG-3′) (17) added to each RT-PCR reaction.

Semiquantitative RT-PCR

Reverse transcription and PCR were performed as described above using the set of primers M3′-S (5′-CTGGATCCTTCTGCAGGACT-3′) and M3′-AS (5′-CCATGCTCGGGAAGTGACT-3′) except that amplification was for 20 cycles and that 1 nCi/ml [32P]dCTP was added per tube. The 424-bp amplification product (nucleotides 2728–3152 of the mouse CIITA cDNA) was resolved on a 6% PAGE and autoradiographed.

RACE-PCR

RACE-PCR assays were performed with the 5′-RACE-PCR kit (Gibco/BRL) according to the manufacturer’s instructions. CIITA cDNAs were obtained by RT-PCR using CM12-AS primer (5′-CTGAGGCTGACACTGTCCTCAG-3′; nucleotides 758–738). TdT-tailing of cDNA was carried out for 1 h at 4°C by incubating 10 μl of the resulting cDNA, 200 μM dCTP, 5 μl 5′ TdT-tailing buffer and 1 μl of TdT. A specificity control reaction was performed in the absence of TdT. For PCR amplification, CM20-AS primer 5′-TGCTGAGTACCTGGTACGAG-3′ (nucleotides 331–308) and Abridged Anchor Primer (AAP; Gibco) were used following the procedures described above. Nested-PCR was performed using 1/10 dilution of the PCR amplification product and CM21AS primer 5′-CCCTGGAGCCACAGCTTAAC-3′ (nucleotides 1160–1131) and Abridged Universal Amplification Primer (AUAP; Gibco).

Sequencing

Sequencing of each RT-PCR product was performed with a DNA sequencer 370A (Perkin-Elmer Cetus). Fluorescent deoxynucleotide labeling of DNA was performed by PCR with the ABI prism kit, following the manufacturer’s instructions (Perkin-Elmer Cetus). The primers used to sequence each RT-PCR product were the same as those used for the RT-PCR experiments.
Immunoprecipitation and Western blot of CIITA

Immunoprecipitation was made with a polyclonal rabbit anti-human CIITA directed against a peptide \(726\text{GEIKDKELPQYLALTR}^{741}\) described in (34) and immunofinity purified through a CNBr-activated Sepharose 4B column (Amersham Pharmacia Biotech, Piscataway, NJ) coupled to the peptide. Western blots were performed with a mAb anti-human CIITA clone 7-1H from R & D Systems (Minneapolis, MN). Cells were incubated with mouse IFN-\(\gamma\) (250 U/ml) and lysed either in RIPA buffer or in high salt buffer (10 mM HEPES, pH 7.9, 0.4 M NaCl, 1.5 mM MgCl\(_2\), 0.1 mM EGTA and 5% glycerol) containing 0.5% NP-40 (Sigma). Lysates were cleared for 2 h with Protein G-Sepharose (Amersham Pharmacia Biotech) and CIITA proteins were next immunoprecipitated overnight with 2.5 \(\mu\)g of affinity-purified polyclonal anti-CIITA antibodies per sample. Immune complexes were recovered by binding for 30 min to Protein G-Sepharose, resolved on a 6% SDS–PAGE and absorbed to a nitrocellulose membrane (Hybond ECL; Amersham Pharmacia Biotech). Blocking was performed overnight in PBS/Tween buffer containing 2.5% low-fat milk. Incubation with 1.5 \(\mu\)g/ml of mAb anti-CIITA was performed for 2 h at room temperature in PBS/Tween buffer containing 0.25% low-fat milk. Immunoreactive bands were visualized with the ECL Plus Western blotting system (Amersham Pharmacia Biotech). To test for proteolytic activity in the RAG cell line, 2 mg of total cell extracts of RAW264.7 and RAG cells was mixed and incubated for 1 h at 37°C with or without proteases inhibitors from Sigma (0.4 mM PMSF, 3 \(\mu\)g/ml aprotinin, 1 \(\mu\)g/ml leupeptin and 0.5 \(\mu\)g/ml pepstatin A) followed by immunoprecipitation as described above. Moreover, the same type of experiments were performed using 10 \(\mu\)M of the proteasome inhibitor NLVS, kindly donated by Dr Hidde Ploegh (Boston, MA).

Northern blot analysis

Total RNA was isolated by the guanidinium thiocyanate procedure. Northern blot analysis was performed as previously described (35). The IFN regulatory factor (IRF)-1 and IRF-2 full-length cDNA probes were kindly provided by Hitoshi Ueda (Hyogo, Japan), and the guanylate binding protein (GBP)-1 cDNA probe was kindly provided by Mark Richter (Paris, France).

Luciferase constructs and assays

Plasmid constructs were made in the pGL3-basic vector from Promega (Madison, WI). The 445 bp pIV promoter fragment was obtained by PCR on mouse genomic DNA from L(tk\(^{-}\)) and RAW264.7 cells using the primer set P1-S (5'\text{GGTTGG-GCTGAGATAGAGTG-3'}\) and P1-AS (5'\text{CTGTGCCCCGT-GCTTCTGAG-3'}\) designed after the published sequence (18). Both PCR fragments were subcloned in the SmaI site of pGL3-basic and sequenced. The resulting constructs were called pIV-L(tk\(^{-}\)).1, pIV-L(tk\(^{-}\)).8 and pIV-RAW5.1. Assays were performed with the luciferase assay system from Promega as described previously (19). The pGL3-control vector contains the SV40 promoter driving the luciferase expression. Transfection efficiency was monitored using the pSV\(\beta\)-galactosidase vector from Promega.

Results

MHC class II-defective RAG and L(tk\(^{-}\)) cell lines display different genetic defects

The RAG and L(tk\(^{-}\)) cell lines do not express MHC class II genes after IFN-\(\gamma\) treatment even though IFN-\(\gamma\) induces MHC class I cell surface expression in both cell lines (Fig. 1). In order to determine if RAG and L(tk\(^{-}\)) display defects in the same gene, RAG x L(tk\(^{-}\)) hybrid cells were generated. These hybrids were analyzed by indirect immunofluorescence for surface MHC class II expression using two allele-specific mAb that allowed us to distinguish between MHC class II molecules from each cell line [haplotypes d and k for RAG and L(tk\(^{-}\)) respectively]. RAG x L(tk\(^{-}\)) hybrids display cell surface expression of both I-A\(^d\) and I-A\(^k\) MHC class II molecules when treated with IFN-\(\gamma\) demonstrating that I-A genes are functional in both cell lines (Fig. 1). Additionally, we conclude from these results that the RAG and L(tk\(^{-}\)) cell lines display mutations in different genes, and that both defects are recessive.
The RAG cell line

Expression of C2ta transcripts in the RAG cell line. We have previously described that MHC class II expression can be restored in RAG cells by transfection of the human CIITA cDNA. However, analysis of hybrid cells between RAG and CIITA-deficient cell lines indicated that C2ta was not mutated in the RAG cell line (26). These data therefore suggest that a factor required for CIITA expression might be missing or defective in these cells.

Defects in MHC2TA transcription associated with a general alteration on the IFN-γ transduction pathway have been described for different tumor cells. Indeed, defects on the transcripts of GBP-1 and MHC2TA were found after IFN-γ induction of the non-small cell lung cancer and small cell lung cancer tumor cell lines (23). We therefore investigated if the IFN-γ transduction pathway was fully functional in the RAG cell line through the analysis of different IFN-responsive gene expression. The hybrid cell lines R.GES2.1 and RR2.16, which express MHC class II molecules after an IFN-γ incubation, were used as positive controls. These somatic cell hybrids were previously described and they were generated by the fusion of RAG cells with two different human cell lines. They contain human chromosome 16 or fragments of this chromosome (R.GES2.1 and RR2.16 respectively) and we have demonstrated that the recovery of mouse MHC class II expression was correlated with the presence of this chromosome (25). The expression of the IRF-1, IRF-2 and GBP-1 IFN-γ-responsive genes was assayed through Northern blot analysis. Figure 2 demonstrates that all three genes are inducible in RAG cells with similar intensities when compared to the somatic cell hybrids. Therefore, the defect in the RAG cell line does not affect the IFN-γ transduction pathway and is limited to the expression of MHC class II molecules.

We next examined the transcription of C2ta in RAG through a semiquantitative RT-PCR assay (20 cycles). As controls we used RNA from mouse CIITA+ (RAW264.7) and CIITA− (J777.4) cell lines. As shown in Fig. 3, RAG cells display a significant amount of C2ta transcripts after induction with IFN-γ. These levels of C2ta mRNA are only slightly weaker than...
sequences of both the RAW264.7 and RAG cDNAs compared to the published sequences are summarized in Table 2. Differences found in the absence of major abnormalities in the splicing of (data not shown). In order to assess the absence of minor detection by Western blot with a mAb directed against the protein. As shown in Fig. 4(A), multiple CIITA forms were detected in the control RAW264.7 cell line treated with IFN-γ for 12–21 h, as previously observed in human cell lines (Barbieri et al., submitted and our unpublished data). However, CIITA was not detected in cell extracts from RAG cells. Following the hypothesis that CIITA might be improperly folded or located in a specific compartment in the RAG cells that would hamper its solubilization in mild detergents, the immunoprecipitation was also performed using RIPA lysis buffer that includes SDS (Fig. 4B). Here again CIITA could not be detected in RAG cell extracts, even when increasing the IFN-γ induction period up to 72 h. These data thereby demonstrate that even though C2ta transcripts are present, the CIITA protein is not detectable in the RAG cell line.

A high abnormal proteolytic activity might account for the lack of CIITA protein in RAG cells. To test this point, we analyzed the stability of the CIITA protein expressed by the RAW264.7 cells in the presence of different amounts of cytosolic extracts from RAG cells prepared using mild detergent conditions known to preserve proteolytic activity (36). These experiments did not reveal any evidence of an enhanced degradation of the CIITA protein by RAG cell extracts (data not shown).

The L(tk−) cell line

Complementation of MHC class II expression. We have already shown that the mouse fibrosarcoma cell line L(tk−) does not express MHC class II molecules after IFN-γ treatment (37). Moreover, transfection of MHC2TA genomic DNA in the L(tk−) cells was shown to restore cell surface I-A expression (17), thereby suggesting that L(tk−) cells are defective in MHC2TA. This led us to study further the defect in MHC class II expression in the L(tk−) cell line.

We prepared several somatic cell hybrids between L(tk−) cells and human B lymphoblastoid cell lines (GES and LM) or with the human amniotic cell line WISH. Out of the 13 hybrid clones and subclones analyzed here, eight were induced by IFN-γ to express mouse MHC class II molecules (Fig. 5 and data not shown). Consequently, human genetic material is able to complement the expression of MHC class II in L(tk−) cells.

It has already been shown that in human × mouse hybrids, human chromosomes are segregated over the mouse genetic background (38). This phenomenon can be used to identify the human chromosomes which allow the restoration of a normal phenotype in mutated mouse cell lines (25,38). However, this methodology could not be applied here as the
hybrids with the L(tk-) background contained multiple fragmented human chromosomes, thereby hampering any significant analysis through karyotyping. Therefore, we analyzed by PCR the segregation of human MHC2TA in the whole panel of somatic cells hybrids described above. Two other genes, encoding protamine I (PRMI) (16p13.13) and adenine phosphoribosyl transferase (APRT) (16q24.2), located on human chromosome 16 were used as markers for this chromosome. Results are shown in Table 3. A positive correlation was evidenced between the presence of human MHC2TA and PRMI and expression of mouse MHC class II molecules. No association was found with other transcription factor encoding genes (RFX5 and RFXAP) described to be mutated in MHC class II-defective cells nor with the APRT gene. These results are in agreement with a mutation in the C2ta gene in the L(tk-) cell line.

Analysis of CIITA expression in the L(tk-) cell line. The analysis of C2ta transcription in the L(tk-) cell line revealed a high degree of complexity. The level of C2ta transcripts was much lower in the L(tk-) cell line than that observed in the RAW264.7 or in the RAG cells (Fig. 6). Indeed, when examining the activity of C2ta promoter IV through luciferase reporter gene experiments, the L(tk-) cells were 4 times less active than the RAW264.7 cells when IFN-γ was used to induce transcription from this promoter (Fig. 7). In contrast, the activity of the SV40 promoter did not present any major difference between both cell lines (Fig. 7, pGL3-control vector).

In addition, depending on the primer set used, the relative level of expression of C2ta transcripts in L(tk-) compared to the RAW264.7 cells was highly variable (Fig. 6). This phenomenon was particularly visible in the 5' region of the CIITA
cDNA in L(tk±) where amplification of the cDNA was not observed with primer sets hybridizing on the first 950 bp of the C2ta transcript. RACE-PCR did not allow the precise determination of an intragenic transcription initiation site. A highly G-rich region and the weak amount of transcripts hampered this methodology. We additionally did not evidence alternative splicing events with transcripts initiated from either promoter III and IV. The sequencing of both alleles of promoter IV of C2ta did not reveal any mutation. Nucleotide sequencing of the CIITA cDNA from L(tk±) cells (nucleotides 952–4989) revealed different substitutions in the coding region compared to the published sequence which are summarized in Table 2. Moreover, a deletion of two out of 14 repetitive motifs (ccaaacccaa) present in the non-coding cDNA sequence was found. Immunoprecipitation of the CIITA protein was next attempted using L(tk±) cell extracts. As seen in Fig. 8, and in agreement with the above observations, the protein could not be evidenced in the L(tk±) cell extracts whatever induction period with IFN-γ (16–48 h) was applied.

Taken as a whole our data indicate that the L(tk±) cell line presents altered C2ta transcripts and is devoid of the CIITA protein.

Discussion

Loss of MHC class II expression has been observed in numerous types of tumor cell lines: small cell lung cancers (39), non-small cell lung cancers, neuroblastoma (40), cervical carcinoma and retinoblastoma cell lines (23), pancreatic tumors (41), certain melanoma cell lines (24), choriocarcinoma (42) or multiple myeloma (43). In the latter two cases MHC class II expression has been shown to be repressed in association with the differentiation state of the cell lines (42,43). In contrast, the other tumor cells were expected to express MHC class II following IFN-γ stimulation. Several of them (neuroblastoma, retinoblastoma or plasmacytoma cell lines, small cell lung cancers and pancreatic tumors) were shown to lack MHC2TA transcription (23,39,40,41). However, the defects causing the lack of MHC2TA transcription have not been established, except for the pancreatic tumor cells and a corresponding cell line, where a mutation in the IRF-2 gene has been evidenced (41). Therefore, we have studied two mouse cell lines presenting a defect in MHC class II expression in order to define more precisely the types of mutations that can affect tumor cells.

Different from the above cell lines, the L(tk±) fibrosarcoma cell line displays a weak amount of C2ta transcripts. Interestingly, defects hampering CIITA expression have been observed at different levels in this cell line. We had previously shown that the L(tk±) cells exhibit a poor induction by IFN-γ of certain IFN-γ-responsive genes (genes 1–8 and (2’-5’)oligoadenylate synthetase) (37), although it is normally responsive for the stimulation of MHC class I molecules (17,37). However, the IFN-α response is unaltered (37), suggesting that this cell line displays a selective and partial defect in the IFN-γ transduction pathway. In agreement with this phenotype, the expression of C2ta promoter IV driving a luciferase reporter gene was reduced 4-fold in the L(tk±) cells when compared with the RAW264.7 cell line (Fig. 7). Defects in the IFN-γ transduction pathway is not a rare phenomenon in tumor cell lines, although to our knowledge these defects usually totally inhibit the IFN-γ response, as shown in cervical carcinoma cell lines (23), pancreatic tumors (41), melanoma (44) or renal cell carcinoma cell lines (45).

In addition to the poor C2ta transcript expression in the L(tk±) cell line, we have shown that C2ta transcripts are truncated of ~950 nucleotides in their 5′ region. RACE-PCR techniques have not allowed the identification of the actual transcription initiation site of these transcripts. They might either originate from illegitimate transcription start site(s) or from an intragenic promoter as suggested in a previous report (19). Based on these data we have attempted to identify a truncated form of the CIITA protein which was not detected (data not shown).

Our data show that transcription from promoter IV of C2ta does not occur in a detectable fashion in the L(tk±) cell line. Data from this report and (17) suggest that the defect of the L(tk±) cell line resides in the C2ta gene, thus we examine the existence of mutations in the L(tk±) C2ta promoter IV. We show here that a 445-bp promoter fragment from L(tk±) drives normal expression of the luciferase reporter gene in the RAW264.7 cell line (Fig. 7) and displays a normal nucleotide sequence (data not shown). The lack of expression from promoter IV might then be explained by mutations outside the minimal promoter. However, it is possible that the C2ta endogenous promoter is not functional due to an epigenetic phenomena. Indeed, in a human choriocarcinoma cell line, methylation of MHC2TA promoter IV has been shown to inhibit MHC2TA transcription and thereby the expression of HLA-D genes (42). Alternatively, binding of histone deacetylases to MHC2TA promoter IV might have similar effects on CIITA expression, as exemplified in a plasmacytoma cell line (40). Blockade of C2ta promoter IV might then favor a residual transcript initiation from an intragenic position or illegitimate transcription initiation(s). In summary, in addition to different mutations (or polymorphisms) in the CIITA cDNA sequence, alterations have been observed in the L(tk±) cell line with a lack of transcription from the promoter IV and partial modifications in the response to IFN-γ.
In contrast, RAG cells have retained the transcription of the C2ta gene after treatment with IFN-γ, even though they lack MHC class II expression. Interestingly, this unusual phenotype has been described in another mouse adenocarcinoma cell line (40). Our data assess that the RAG cell line is not defective for the C2ta gene and that the IFN-γ transduction pathway is functional. In addition, the cDNA CIITA nucleotide sequencing has confirmed the absence of mutation in the coding region and the lack of splicing defects. RACE-PCR experiments have demonstrated that transcription initiation occurs within sites that have been described previously in normal cell lines. All together our data show that the C2ta transcript of the RAG cell line is expressed and that it is devoid of defects. However, we have not shown that the factors required, downstream of CIITA, for MHC class II gene expression were functional in this cell line (35).

We have shown in this report that the CIITA protein is not detected in the RAG tumor cell line, in agreement with the lack of MHC class II transcription and cell surface expression. Different hypotheses are compatible with our data. One possibility is that the C2ta transcript is not translated either through a blockade of its nuclear export or through the lack of a factor required for the translation of CIITA. It is also possible that the CIITA protein is expressed; however, in such a labile manner that we did not evidence this protein. Data obtained on cells infected with Chlamydia showed that this organism expresses a protease degrading RFX5 and up-stream stimulatory factor (USF)-1 (36,46), and thereby inhibits MHC class II expression in the infected cells. As the RAG cell line could be infected by an oncogenic virus presenting similar capacities, a highly active viral protease might degrade the CIITA protein. This phenomenon was not evidenced in this report, in agreement with the recessivity of the RAG mutation that does not fit with this hypothesis. We also hypothesized that CIITA might be rapidly degraded by an over-active immune proteasome in the RAG cell line. However, treatment of these cells with a proteasome inhibitor did not alter MHC class II expression, although it reduced MHC class I expression (data not shown). This suggests that C2ta and MHC class II gene-specific transcription factors are not submitted to proteasome degradation. One additional possibility is that a specific chaperone, allowing the proper intracellular trafficking or editing of the CIITA protein, is inactive or missing in the RAG cells. Interestingly, we have shown that the transfection of a
human CIITA cDNA allows for the immunoprecipitation of the human CIITA protein (data not shown) and restores MHC class II expression. This might indicate that the over-expression of a recombinant form of CIITA bypasses these putative defects or that the missing or mutated factor is not required for the human form of the CIITA protein or transcript. Even though we have shown that both the C2ta gene and transcript are normal in the RAG cell line, numerous hypotheses remain concerning the defect on these cells, as this defect can affect various stages of CIITA protein expression as proposed above. Further study of this cell line will therefore help in the understanding of CIITA synthesis and MHC class II expression.

The importance of MHC class II molecule expression in tumor cells is quite controversial (21,47,48). The loss of CIITA expression might therefore confer a selective advantage to tumors from certain cell types. This class II molecules is not essential with regard to immune escape. The loss of CIITA expression might therefore alter tumor growth, tumor progression or immune escape. Recent data suggest that the lack of CIITA itself might be the crucial step. Indeed CIITA, through the sequestration of NFAT (49,50), has been shown to modulate the expression of NF-κB. CIITA can interact with cyclins (51) or the transactivator CBP (11). One can therefore hypothesize that CIITA, through an indirect effect, might alter tumor growth, tumor progression or immune escape. The loss of CIITA expression might therefore confer a selective advantage to tumors from certain cell types. This thereby reinforces the necessity of the identification of the defects altering CIITA expression in tumor cell lines and the precise targets of CIITA effects.

Acknowledgements

This research was supported by the Association pour la Recherche sur le Cancer, INSERM, Fondecyt (8000011) and EcosConicyt (C99S02). R. N. was supported by a grant from Fondecyt (4980004 and 2950018). G. B. was supported by a grant from INSERM and Foundation of France. A. M. L. was supported by a fellowship from the Ligue Nationale contre le Cancer. V. D. was supported by a grant from the Association pour la Recherche sur le Cancer.

Abbreviations

APC antigen-presenting cell
CBP CREB binding protein
CIITA class II transactivator
CREB cAMP-responsive element binding protein
GBP guanylate binding protein
IRF IFN regulatory factor
NFY nuclear factor Y
RFX regulatory factor X
USF up-stream stimulatory factor

References

Defective CIITA expression in tumor cells