A Novel Antigen-processing–defective Phenotype in Major Histocompatibility Complex Class II–positive CIITA Transfectants Is Corrected by Interferon-γ

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Summary

Presentation of exogenous protein antigens to T lymphocytes is based on the intersection of two complex pathways: (*a*) synthesis, assembly, and transport of major histocompatibility complex (MHC) class II–invariant chain complexes from the endoplasmic reticulum to a specialized endosomal compartment, and (*b*) endocytosis, denaturation, and proteolysis of antigens followed by loading of antigenic peptides onto newly synthesized MHC class II molecules. It is believed that expression of MHC class II heterodimers, invariant chain and human leukocyte antigen–DM is both necessary and sufficient to reconstitute a functional MHC class II loading compartment in antigen-presenting cells. Expression of each of these essential molecules is under the control of the MHC class II transactivator CIITA. Unexpectedly, however, whereas interferon γ stimulation does confer effective antigen-processing function to nonprofessional antigen presenting cells, such as melanoma cells, expression of the CIITA transactivator alone is not sufficient. Activation of antigen-specific T cells thus requires additional CIITA-independent factor(s), and such factor(s) can be induced by interferon γ.

The MHC class II transactivator CIITA has been described as a transcription factor necessary for the regulation of both constitutive (1) and IFN-γ–induced (2) expression of MHC class II genes. CIITA transfection into various human cell lines was found to be able to induce surface expression of MHC class II molecules to levels similar to those induced upon IFN-γ treatment (2).

This essential role of CIITA suggested that MHC class II–negative, nonprofessional APC could be converted by CIITA transfection into MHC class II–positive cells able to activate antigen-specific, class II–restricted T cells. The potential interest of this approach in the field of immunotherapy prompted us to address this possibility. We present here the functional analysis of CIITA-transfected human melanoma cells acting as APC for HLA-DR–restricted CD4$^+$ T lymphocytes. CIITA transfection is able to confer antigen presentation function when cells are provided with peptide antigen, but, unexpectedly, the same transfectants remain deficient in processing of protein antigens. The analysis of the structural and functional characteristics of CIITA transfectants identified an antigen-processing defect that exhibits a novel phenotype. Correction of this antigen-processing defect by IFN-γ treatment indicates that factor(s) other than MHC class II, invariant chain (Ii),[1] and DM, which are all induced by CIITA expression are necessary for optimal processing and presentation by nonprofessional APC such as melanoma cells.

Materials and Methods

Cells and Culture Conditions. Human melanoma cells Me67 and Me208 were grown in RPMI-1640 medium complemented with glutamine, 10% heat-inactivated (56°C) FCS, and antibiotics. Cells were incubated at 37°C in 5% CO_2 and maintained in a logarithmic growth phase with a viability >98% at all steps. For MHC class II induction, cells were incubated with human rIFN-γ (specific activity = 1.4×10^7 U/mg; gift from Biogen Inc., Cambridge, MA) at 500 U/ml for 24 or 48 h, as indicated in figure legends.

Transfections. Melanoma cell lines were transfected by calcium phosphate precipitation followed 4 h later by a glycerol shock with either the expression vector EBO-Sfi alone or a full-length CIITA cDNA cloned into EBO-Sfi under control of SV40 promoter (1, 2). Stable transfectants were generated by selection with hygromycin B (Calbiochem Corp., La Jolla, CA) and maintained in culture with hygromycin throughout the study, including during IFN-γ induction.

Surface MHC Class II Expression. Duplicate samples of 2×10^5 cells were washed, preadsorbed with 10% normal rabbit serum (NRS), incubated with NRS or relevant antibodies followed by fluorescein-conjugated rabbit anti–mouse IgG (Serotec Ltd., Oxford, UK), washed, and analyzed by flow cytometry on a FACScan® analyzer (Becton Dickinson & Co., Mountain View, CA). 10,000 cells were analyzed for each determination.

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[1] *Abbreviations used in this paper:* HA, hemagglutinin; Ii, invariant chain; NRS, normal rabbit serum; OVN, overnight.

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Antibodies. Polymorphic HLA-DR mAB 2.06 (3), mAb GSP-142.2 (Genetic System Corp., Seattle, WA), GSP87.1 (id.), and mAbs M2, 16.23, A2, and KS.6 (4) were used at saturating concentrations for indirect immunofluorescence and immunoprecipitation (mAb D1.12; reference 5) studies.

SDS Stability of MHC Class II Dimers. Immunoprecipitation of MHC class II molecules was done as described (5). Briefly, 10×10^6 cells per point were labeled for 8 h at 37°C with 500 μCi of $[^{35}S]$methionine (Amersham Corp., Arlington Heights, IL) in 5 ml of RPMI medium lacking methionine and containing 2% dialyzed FCS. Cells were then washed extensively in cold PBS before lysis fo 20 min at 4°C in 1 ml of Tris-buffered saline containing 1% NP-40 detergent (5). Insoluble material was removed by centrifugation at 100,000 *g* for 30 min. Cell extracts were incubated three times for 2 h at 4°C with protein A–Sepharose 4 Fast Flow beads (Pharmacia, Uppsala, Sweden) under rotation to remove nonspecifically bound material. Class II molecules were then immunoprecipitated by using mAb D1.12, previously bound to protein A–Sepharose beads, for 4 h at 4°C. Cell extracts were then washed three times in Tris-buffered saline with 0.5% NP-40. Samples were resuspended in 2% SDS buffer containing 10% glycerol and split in two portions, one of which was boiled for 5 min and one of which was left at room temperature for 30 min. Samples were separated on 10.5% polyacrylamide gels before autoradiography.

T Cell Lines. T-19 is a T cell line specific for the p2 peptide of tetanus toxin (tt830-843), which is restricted by the DRB1*11 and DRB1*08 alleles (6), whereas T-87 recognizes the p4 peptide (tt 1273-1284) presented by DRB3*0101 (data not shown). T cell lines were generated as described (6). RPMI-1640 supplemented with 15% human AB^+ serum from male volunteer donors was used as culture medium. T cell lines were restimulated for expansion with autologous irradiated PBMC, preincubated with p2 or p4 peptides, in IL-2–supplemented culture medium. Expanded T cell lines were frozen in culture medium–DMSO 10% and stored in liquid nitrogen.

Antigen Presentation to T Cells. Melanoma cells (10^6) were incubated for 16 h with various concentrations of tetanus fragments Tet3 (tt 744-1315, reference 7), C (Fr-C, tt 865-1315), B (Fr-B, tt 2-864), or medium alone, fixed with 0.2% paraformaldehyde, washed, and used as APC (3×10^4 cells/well) in coculture with tetanus-specific T cell lines (2×10^4 cells/well) as described (6). Alternatively, melanoma cells were fixed and preincubated with various concentrations of tetanus p4 or p2 synthetic peptides before washing and coculture with T cells. The proliferative response of tetanus-specific T cell lines was measured after 48 h by $[^3H]$thymidine incorporation as described (6).

Peptide Binding. Binding assays were performed as described (8). Briefly, 3×10^5 melanoma cells, either CIITA transfected or IFN-γ treated, were incubated at 37°C for 4 h with various concentrations of a biotinylated peptide (HA 307-319) of influenza hemagglutinin (HA) (9) or medium alone. In competition experiments, cells were first incubated with a large excess of nonbiotinylated HA peptide. After washing, cells were incubated with FITC-streptavidin 4.22 μg/ml, Calbiochem Corp.) at 4°C for 30 min. Stained cells were washed again and analyzed by flow cytometry as described above.

Results

Antigen Presentation Function of CIITA Transfectants. CIITA-transfected or IFN-γ–treated Me67 and Me208 melanoma cells were first compared by indirect immunofluorescence analysis for their level of surface HLA-DR molecules. Similar staining intensity by HLA-DR–specific mAbs were observed under both conditions in both cell lines (Fig. 1 *A*), confirming our previous findings on the role of CIITA on MHC class II induction (2). Profiles of cells transfected with the EBO-Sfi vector alone were superposable with those of untransfected, noninduced cells (data not shown). Furthermore, the rate of synthesis of HLA-DR molecules is identical in CIITA-transfected or IFN-γ–treated Me67 cells (see below).

We next assessed the functional capacity of CIITA-transfected or IFN-γ–treated MHC class II–positive cells to present tetanus toxin antigen to tetanus-specific T cell clones.

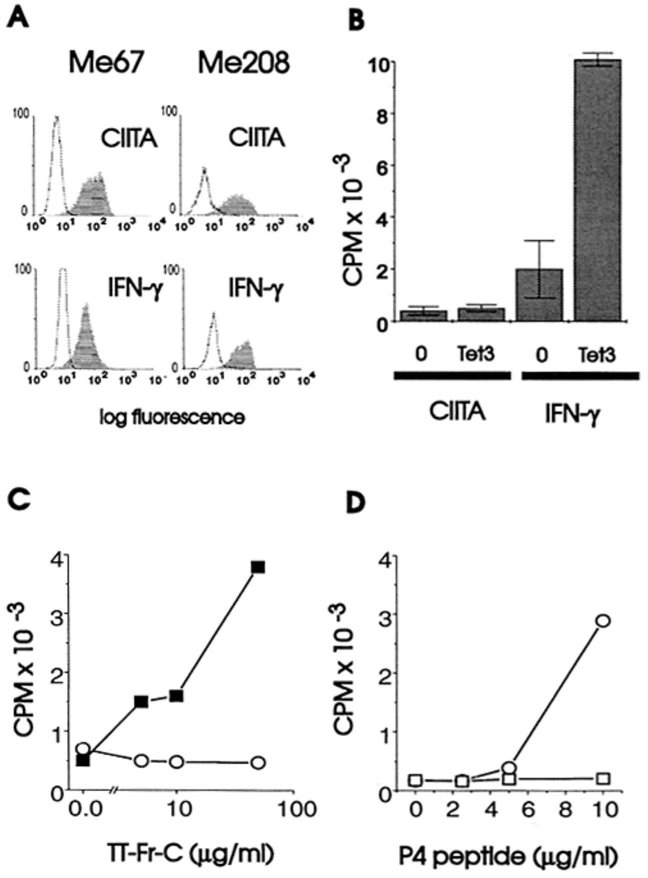

Figure 1. Antigen presentation by CIITA-transfected or IFN-γ–treated melanomas. (*A*) Surface expression of HLA-DR on Me67 and Me208 cells transfected with CIITA or incubated with 500 U/ml of IFN-γ for 48 h. Cells were analyzed by flow cytofluorimetry for the expression of HLA-DR (mAb 2.06). Blank profiles on the left represent cells incubated with NRS and the second reagent alone. (*B*) Specific recognition by T-19 T cells of exogenous tetanus toxin presented by Me208 cells transfected with CIITA or incubated with 500 U/ml of IFN-γ for 24 h before OVN incubation with 10 μg/ml of tetanus fragment Tet3 (tt 744-1315, ref 5) or medium alone. (*C*) Specific recognition of exogenous tetanus toxin presented by Me67 cells. IFN-γ–treated (–■–) or CIITA transfected (–○–) cells were incubated OVN with various concentrations of tetanus toxin fragment C (Fr-C, tt 865-1315) before fixation and coculture with T-87 cells. (*D*) Presentation of exogenous synthetic peptide by CIITA-transfected Me67 cells. CIITA-transfected (–○–) or control Me67 cells (–□–) were fixed and preincubated OVN with various concentrations of p4 synthetic peptide before washing and coculture with T-87 cells.

Me208 cells that were either CIITA transfected or preincubated with 500 U/ml of IFN-γ for 24 h were incubated overnight (OVN) with 10 μg/ml of tetanus fragment Tet 3, fixed, and used as APC in coculture with the tetanus-specific T-19 T cell line (Fig. 1 *B*). This T cell line, specific for the p2 peptide of tetanus toxin (tt 830-843), is restricted by the DRB1*11 and DRB1*08 alleles (6). IFN-γ induction of MHC class II molecules readily conferred to Me208 melanoma cells expressing DRB1*1101/04 alleles (oligotyping, data not shown) the capacity to activate T-19 lymphocytes. In contrast, CIITA-transfected Me208 expressing similar amounts of MHC class II molecules (Fig. 1 *A*) were unable to activate the T-19 cell line. This dramatic difference in antigen presentation capacity between IFN-γ–induced and CIITA-transfected cells was also observed under similar conditions with a different melanoma cell line (Me67, DRB1*1301/04, DRB3*0101) presenting a different epitope (peptide p4, tt 1273-1284) to a T cell line restricted by a different HLA molecule, the DRB3*0101-restricted T-87 cell line (Fig. 1 *C*).

In contrast, when the same CIITA-transfected melanoma cells unable to process and present native tetanus protein antigen to T cells were incubated with various concentrations of synthetic tetanus peptides, a dose-dependent peptide-specific DR-restricted activation of T cell lines was induced (Fig. 1 *D*). Thus, CIITA transfection of class II–negative melanoma cells induces a normal expression of surface MHC class II molecules that are able to bind and present exogenous peptides to specific, DR-restricted T cells. Curiously, however, it creates an antigen-processing–deficient phenotype.

Stability of MHC Class II Dimers in CIITA Transfectants. The description of HLA-DM mutant B cell lines characterized by impaired processing of exogenous native antigens, loss of MHC class II SDS stability at room temperature, and expression of distinct MHC class II conformational epitopes (10–14) prompted us to first address the possibility of a similar phenotype in CIITA transfectants. To analyze the SDS stability of their class II dimers, CIITA-transfected or IFN-γ–treated Me67 cells were labeled with $[^{35}S]$methionine before immunoprecipitation of MHC class II molecules with mAb D1.12. Samples resuspended in 2% SDS buffer were split into two portions, one of which was boiled for 5 min and one of which was left at room temperature for 30 min before electrophoresis (Fig. 2 *A*). Immunoprecipitation of newly synthesized HLA-DR molecules in either CIITA-transfected or IFN-γ–treated Me67 cells demonstrated (*a*) a similar rate of HLA-DR biosynthesis and associated Ii, and (*b*) a similar pattern of SDS stability of class II dimers. Thus, in contrast to what was observed in HLA-DM–mutant B cell lines, the antigen-processing defect of CIITA transfectants does not prevent the formation of SDS-stable class II dimers.

The induction of both HLA-DM and Ii chains by CIITA transfection has been documented (15, 16) in all cell types examined, and specifically confirmed in the melanoma cell lines used in this study. In the CIITA-transfected or IFN-γ–treated Me67 melanoma cells, the steady-state mRNA ratio of HLA-DR/Ii/DM is strictly conserved quantitatively (RNase protection analysis, data not shown). In terms of intracellular localization of HLA-DR molecules, no differences were observed by confocal microscopy between CIITA-transfected or IFN-γ–treated Me67 cells (data not shown).

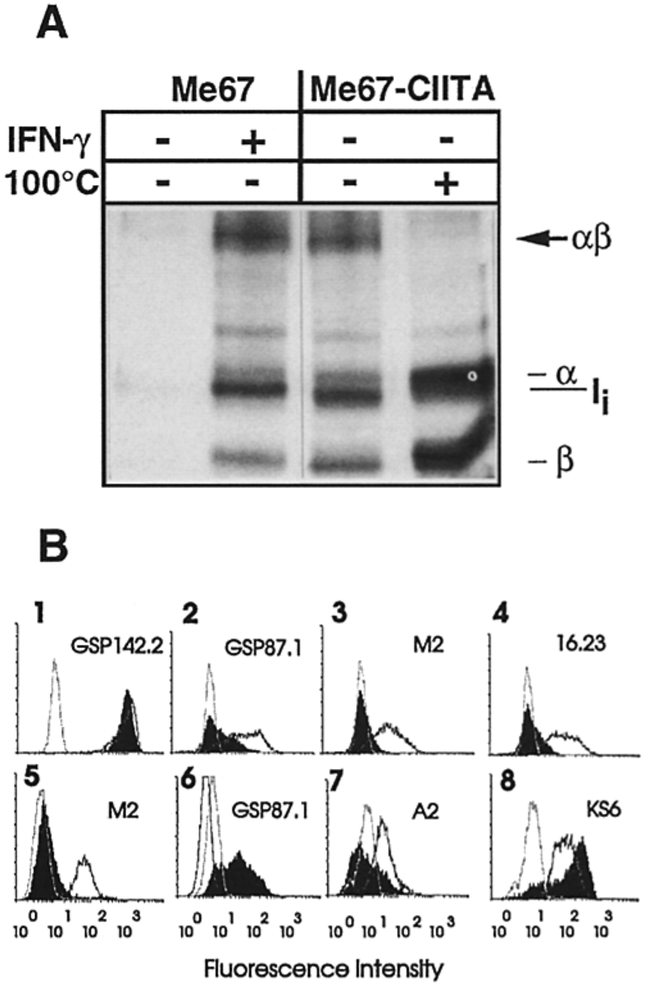

Figure 2. Conformation of MHC class II dimers in CIITA-transfected or IFN-γ–treated melanoma cells. (*A*) Formation of SDS-stable MHC class II dimers in Me67 cells treated (+) or not (−) with IFN-γ and in Me67–CIITA cells. Class II molecules immunoprecipitated in SDS sample buffer were either boiled (100°C) or left at room temperature before separation on SDS-PAGE and autoradiography. αβ, DR heterodimers; α, DRα monoamers; β, DRβ monomers. (*B*) Different conformation of MHC class II molecules at the surface of melanoma cells. CIITA-transfected (*blank*) or IFN-γ–treated (*black*) Me67 cells (*1–4*) and Me108 cells (*5–8*) were analyzed by flow cytofluorometry for the expression of HLA-DR (*1–6*) or -DQ (7 and *8*) with a panel of MHC class II–specific mAbs (18). Blank profiles on the left (*dotted line*) represent melanoma cells incubated with NRS and the second reagent alone.

Surface Conformation of MHC Class II Molecules. The altered conformation of surface MHC class II molecules has been described as another phenotypic feature of antigen-processing–deficient mutants (17–19). Surface MHC class II conformation was therefore studied in CIITA-transfected or IFN-γ–treated Me67 cells expressing strictly similar amounts of MHC class II molecules when stained with

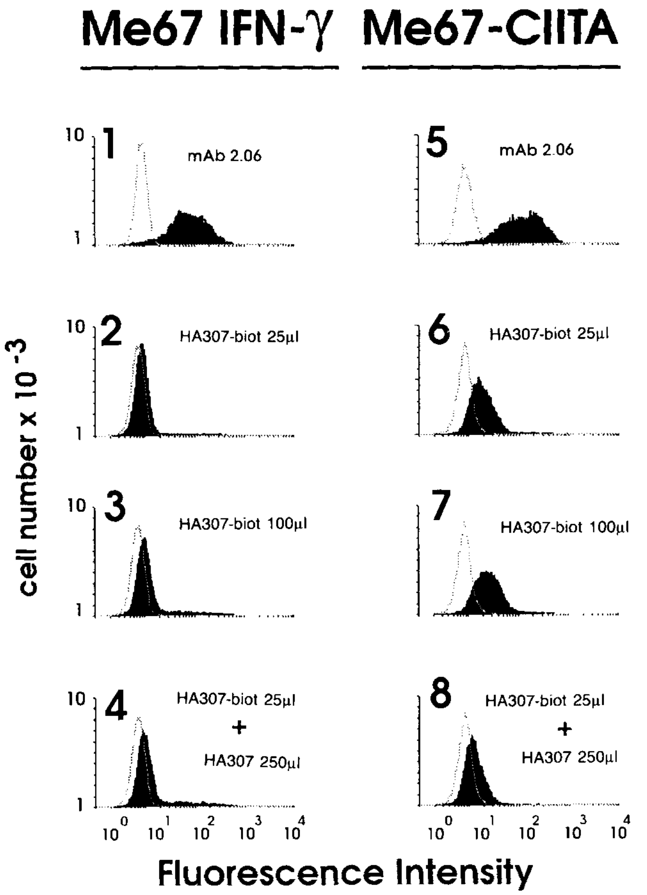

Figure 3. Peptide binding by surface MHC class II molecules. Me67 cells that were either IFN-γ treated (*1–4*) or CIITA transfected (*5–8*) were analyzed by flow cytofluorometry for surface expression of HLA-DR (mAb 2.06, *1* and *5*) or incubated with the indicated concentrations of biotinylated HA307-319 peptide before staining with FITC–streptavidin and analysis by flow cytofluorometry. Profiles on the left indicate cells incubated with the second reagent alone. In competition experiments (*4* and *8*), cells were first incubated with an excess of nonbiotinylated HA307-319 (250 µM) before addition of biotinylated peptide.

polymorphic HLA-DR mAb (Fig. 1 *A* and Fig. 2 *B*, *1*). Out of a large panel of anti-DR, -DP, and -DQ mAbs selected for their relative specificity for a given HLA haplotype, a few antibodies consistently distinguished HLA-DR or HLA-DQ molecules expressed on the same melanoma cell line that was either CIITA transfected or INF-γ treated (Fig. 2 *B*, *2–8*). Certain mAbs such as KS6 (Fig. 2 *B*, *8*) recognized INF-γ–treated cells 10 times better than CIITA transfectants. Other mAbs, such as M2 and 16.23, clearly recognized a determinant of MHC class II molecules at the surface of HLA-DR13 (Me67)$^+$ or HLA-DR11 (Me208)$^+$ CIITA, but not IFN-γ–treated cells (Fig. 2 *B*, panel *4*). Last, depending on the cell haplotype, mAb GSP87.1 recognized an HLA-DR epitope of CIITA-transfected (Me67) cells or IFN-γ–induced (Me208) cells (Fig. 2 *B*, *2* and *6*). No direct correlation was thus found across haplotypes between expression of a given epitope and antigen-processing capacity. However, the distinction between MHC class II molecules induced at the surface of the same cell lines by CIITA transfection or by IFN-γ stimulation indicated that these molecules, present in similar amounts, adopt different surface conformations. These different conformations could well reflect the intracellular binding of different sets of peptides by MHC class II molecules synthesized under both conditions.

Peptide-binding Capacities of Surface MHC Class II Molecules. The adherent phenotype and relatively stringent culture requirements of our melanoma cell lines did not allow culture of a number of cells sufficient for direct elution of MHC class II bound peptides. Nevertheless, to explore the possibility that different sets of peptides could be present within the groove of surface class II dimers in the two conditions, we assessed the capacity of surface class II molecules to bind an exogenously provided synthetic peptide. CIITA-transfected or IFN-γ–induced Me67 cells expressing similar levels of surface class II molecules (Fig. 3, *1* and *5*) were incubated with a biotinylated influenza peptide (HA 307-312) known to bind to HLA-DR4 (9) before addition of FITC–streptavidin and analysis by flow cytometry. Surprisingly, the patterns of fluorescence observed were markedly different. Whereas little binding above autofluorescence was detected for IFN-γ–treated cells, which is consistent with binding by <2% of surface class II molecules, Fig. 3 shows a strong, dose-dependent increase of fluorescence of CIITA transfectants. Competition experiments in which cells were first incubated with a 10× excess of nonbiotinylated peptide (Fig. 3, *4* and *8*), as well as blocking experiments with MHC class II–specific antibodies (data not shown), confirmed the specificity of the fluorescence. Thus, the distinct conformation of MHC class II molecules expressed at the surface of CIITA-transfected melanoma cells correlates with an increased peptide-binding capacity when compared with class II dimers of IFN-γ–treated cells.

Correction of the Antigen-processing Defect of CIITA Transfectants by IFN-γ. The fact that IFN-γ induction was able to confer antigen-processing capacity to melanoma cells, whereas CIITA transfection was not, suggested that the processing defect of CIITA transfectants could result from the lack of expression of other IFN-γ–inducible essential factor(s). The effect of IFN-γ on the antigen-processing ability of CIITA transfectants was therefore studied. Time course experiments, where CIITA-transfected Me67 cells were incubated with IFN-γ for various periods of time before incubation with antigen and specific T cell lines, demonstrated IFN-γ–dependent restoration of tetanus toxoid presentation by CIITA transfectants (Fig. 4). This was not accompanied by an increase in cell surface HLA-DR expression (data not shown). A similar restoration of antigen presentation was obtained with T cell lines specific for other tetanus toxin epitopes, restricted by different HLA-DR alleles, and with the Me208 melanoma cell line (data not shown). These results demonstrate that additional factor(s) induced by IFN-γ, but not under the control of CIITA, are absolutely required to confer antigen-processing capacity to these nonprofessional APC.

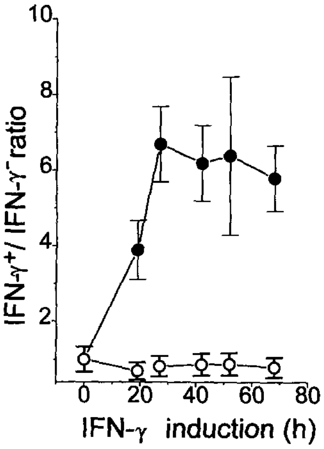

Figure 4. IFN-γ restoration of antigen processing by CIITA-transfected melanoma cells. CIITA-transfected Me67 cells were incubated with 500 U/ml of IFN-γ or medium alone, in continuous presence of hygromycin, for various periods of time before OVN incubation with tetanus toxin Fr-C (20 μg/ml) or control antigen (tetanus toxin Fr-B, tt 2-864, 20 μg/ml). The proliferative response of the T-87 cell line to Fr-C (–●–) or control antigen (–○–) is expressed as the ratio of stimulation obtained with IFN-γ–treated versus–untreated cells.

Discussion

Although expressing similar levels of surface HLA-DR molecules and synthesizing HLA-DR at similar rates, melanoma cells are either capable (IFN-γ treatment) or incapable (CIITA transfection) of presentation of a protein antigen to the relevant T lymphocytes (Fig. 1). In contrast to these results with protein antigens, CIITA-transfected melanoma cell lines provided with the antigen in the form of exogenous synthetic peptides readily activate specific T lymphocytes. Thus, CIITA transfection of these nonprofessional APC induces the expression of MHC class II molecules, confers the capacity of presenting short exogenous peptides to T cells, but generates a cell phenotype that is defective in antigen processing and presentation of native antigens. This unusual functional phenotype, which differs from that of the same cell line after stimulation by IFN-γ, suggests that antigen processing by nonprofessional APC such as melanomas involves yet an additional level of complexity that is not under the control of the MHC class II transactivator CIITA.

Studies of HLA-DM–mutant B cell lines have identified a special phenotype of APC characterized by impaired processing of some exogenous antigens. Consequently, the phenotypical features characteristic of HLA-DM mutants were studied in CIITA-transfected melanoma cells. Immunoprecipitation of newly synthesized HLA-DR molecules in CIITA-transfected and IFN-γ–induced cells demonstrated a similar pattern of SDS stability of class II dimers (Fig. 2 *A*). We conclude that, contrary to the situation of the HLA-DM mutants, the antigen-processing defect of CIITA transfectants does not prevent the formation of SDS-stable class II dimers. The recent demonstration that the formation of class II SDS-stable dimers requires both Ii and DM expression (20) also argues for a distinct mechanism responsible for the antigen-processing defects of CIITA transfectants and HLA-DM mutants. In addition, induction of both HLA-DM and Ii mRNA upon CIITA transfection has been demonstrated (15, 16). Since trace amounts of DM are reported sufficient to stabilize MHC class II molecules (20), and since the DR/Ii/DM mRNA ratio is identical in CIITA-transfected and IFN-γ–induced cells, these factors known to be involved in class II–restricted antigen presentation do not seem to be responsible for the antigen-processing defect observed in CIITA transfectants.

MHC class II molecules expressed on certain processing-defective B cell mutants were shown to exhibit special conformational determinants that can be distinguished by certain mAbs (17–19). This characteristic feature is also present in antigen-processing–deficient CIITA-transfectants, whose surface MHC class II dimers exhibit a different conformation when compared with class II molecules expressed after IFN-γ induction. In the case of HLA-DM–deficient mutant B cell lines, as in CIITA transfectants, the loss of a few conformational epitopes present on normal cells has been observed. In our search for mAbs able to distinguish specific conformational determinants, we also identified mAbs that recognized MHC class II molecules better or exclusively on antigen-processing–deficient cells (Fig. 2 *B*). No direct correlation was found across HLA haplotypes between expression of a given epitope and antigen-processing capacity. Thus, the change in class II conformation that is associated with the antigen-processing defect of CIITA transfectants resulted either in enhanced or in reduced recognition by specific mAbs. Altogether, these analyses suggest, as was described for murine intestinal cells (21) and thymic medulla epithelial cells (22), that the conformation of MHC class II molecules at the cell surface reflects the set of peptides bound within their groove, and thus indirectly the integrity of the antigen-processing pathway. In the case of nonprofessional APC, such as melanomas the demonstration of a distinct structural phenotype appears to correlate with antigen-processing capacity better than SDS stability of class II dimers.

The structural differences of surface class II MHC molecules expressed on CIITA transfectants were further substantiated by the demonstration of a very different capacity for peptide binding. It was indeed of interest to observe a stronger binding of an influenza-derived biotinylated peptide to class II molecules expressed at the surface of CIITA-transfected versus IFN-γ–treated melanoma cells (Fig. 3). Given the similar density of MHC class II molecules expressed under both conditions, this observation strongly suggests an increased avidity of MHC class II dimers of CIITA transfectants for exogenous peptides. The expression of stable MHC class II molecules with distinct conformations and enhanced binding capacity for peptides suggests that MHC class II molecules synthesized in CIITA transfectants do encounter and bind different sets of peptides on their way to the cell surface.

The fact that IFN-γ can correct the antigen-processing–defective phenotype of CIITA-transfected melanomas is important (Fig. 4). It means that in addition to the genes regulated by CIITA (i.e., MHC class II, HLA-DM, and Ii), one or more other INF-γ–induced genes are required for antigen processing in some nonprofessional APC. This is in contrast to what is observed in various mutant B cell lines, where antigen-processing defects can be restored simply by expression of MHC class II, HLA-DM, and Ii (11, 12, 14). We thus suggest that these essential additional factors are

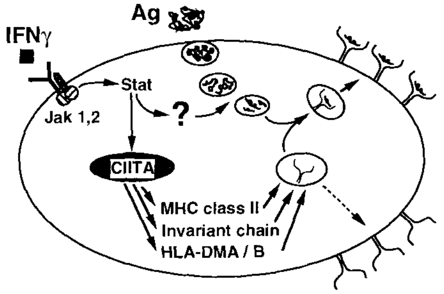

Figure 5. Complexity of antigen-processing and presentation by MHC class II molecules in nonprofessional APC.

not constitutively expressed in nonprofessional APC such as melanomas and that they require cytokine induction. In these nonprofessional APC, it has been reported that the presence of HLA-DM and the Ii chain is sufficient to allow MHC class II molecules to reach the appropriate peptide-loading compartment (20).

It follows that additional distinct steps should be considered as candidates for the essential IFN-γ–inducible, CIITA-independent function required for antigen presentation. Endocytosis does not seem to be involved since kinetic analysis of fluid-phase endocytosis through flow cytofluorometry (23) indicated an identical level of Lucifer yellow uptake for both untreated (processing-defective) and IFN-γ–treated (processing-competent) CIITA-transfected cells (data not shown). Interestingly, there is recent evidence that IFN-γ induces endosomal and lysosomal proteases, such as cathepsin D and B (24–26), which are involved in the processing of endocytosed proteins, including tetanus toxin (27). Thus, the modulation by IFN-γ of the activity or localization of specific proteases could influence the nature of the peptides generated and/or loaded onto MHC class II molecules.

We propose that effective processing and presentation of exogenous antigens by melanoma-like nonprofessional APC not only requires the expression of the various genes controlled by the transactivator CIITA (namely, MHC class II genes, HLA-DM, and the Ii gene), but also depends on the induction, by IFN-γ, of other factors required for processing of protein antigens. Whether these factors result in subtle modifications in postendocytic trafficking steps, in the availability or activity of specific proteases, or in other yet unknown protein–protein interactions is now open for study (Fig. 5). The demonstration that antigen-processing–defective cells of any desired HLA-DR haplotype can be obtained simply by CIITA transfection of nonprofessional APC should now greatly facilitate the search for these factors.

Whatever the exact mechanism, this observation could have practical implications in the field of cancer immunotherapy: transfection of the CIITA gene is indeed considered a way to convert tumor cells into efficient MHC class II–positive APC. This way might not be sufficient for induction of T cells that are specific for tumor antigens. Furthermore, the identification of an additional requirement for antigen processing by nonprofessional APC that express MHC class II molecules, and its control by IFN-γ, appears important in terms of the physiological regulation of immune responses. Indeed, the inability of nonprofessional APC throughout the body to adequately process exogenous antigens would limit the risk of inducing immune responses to numerous environmental antigens. As a significant example, enterocytes have been shown to constitutively express MHC class II molecules of abnormal conformation and to be defective in T cell activation (21). In contrast, in the context of local inflammation, cytokines released in the microenvironment would simultaneously activate MHC class II expression and antigen-processing mechanisms, thus resulting in effective antigen presentation.

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