Results in Immunology 2 (2012) 174–183

Contents lists available at SciVerse ScienceDirect

Results in Immunology

journal homepage: www.elsevier.com/locate/rinim

Contrasting effects of IFNα on MHC class II expression in professional vs. nonprofessional APCs: Role of CIITA type IV promoter

Laura Pisapia[a,1], Giovanna Del Pozzo[a,1], Pasquale Barba[a], Alessandra Citro[b,2], Paul E. Harris[b], Antonella Maffei[a,b,*]

[a] Institute of Genetics and Biophysics A. Buzzati-Traverso, CNR, Naples, Italy
[b] Department of Medicine of Columbia University Medical Center, New York, NY, USA

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form 16 September 2012
Accepted 19 September 2012

Keywords:
Non-professional antigen presenting cells
CIITA promoter IV
IFNα
MHCII

ABSTRACT

We previously demonstrated that, in *ex vivo* cultures, IFNα downregulates the expression of MHC class II (MHCII) genes in human non-professional APCs associated with pancreatic islets. IFNα has an opposing effect on MHCII expression in professional APCs. In this study, we found that the mechanism responsible for the IFNα-mediated MHCII's downregulation in human MHCII-positive non-professional antigen presenting human non-hematopoietic cell lines is the result of the negative feedback system that regulates cytokine signal transduction, which eventually inhibits promoters III and IV of CIITA gene. Because the CIITA-PIV isoform is mostly responsible for the constitutive expression of MHCII genes in non-professional APCs, we pursued and achieved the specific knockdown of CIITA-PIV mRNA in our *in vitro* system, obtaining a partial silencing of MHCII molecules similar to that obtained by IFNα. We believe that our results offer a new understanding of the potential significance of CIITA-PIV as a therapeutic target for interventional strategies that can manage autoimmune disease and allograft rejection with little interference on the function of professional APCs of the immune system.

1. Introduction

The functioning of the immune response in infection, transplantation, cancer and autoimmunity is strictly dependent on the level of expression of MHC molecules on the surface of APCs [1]. Any degree of alterations in expression levels of MHC may influence various events downstream of TcR engagement [2,3]. On the basis of their potential for antigen presentation to T cells, APCs are frequently classified into two major categories: professional or non-professional. Professional APCs have been identified as cells of hematopoietic origin specialized in the priming of naive T cells. These cells, including dendritic cells (DCs), B lymphocytes, and cells of the monocyte/macrophage lineage, can induce both primary and memory immune responses because of their constitutive expression of MHC class II (MHCII) molecules and potent costimulatory molecules. Non-professional APCs have been identified as non-bone marrow-derived cells that do not express a complete range of costimulatory molecules. This definition applies to cell types that do not express basal levels of MHCII molecules but can be induced to express MHCII molecules in response to IFNγ [4], as well as to cell types that constitutively express MHCII molecules, such as thymic epithelial cells [5] and endothelial cells in various organs [6–8]. Spurious expression of MHCII molecules on non-bone marrow-derived cells has also been described in tumor cells from several neoplastic tissues, including glioma and melanoma [9–11]. Finally, the rejection of transplanted organs strictly depends on the MHCII expression in endothelial and epithelial cells in the transplant and in the host tissues [12].

MHCII expression is mainly regulated at the level of transcription by CIITA [4,13], a non-DNA-binding factor that exhibits a cell-type-specific, cytokine-inducible and differentiation-stage-specific expression profile [14]. In humans, four different CIITA transcription products have been identified, each of which is generated by one independent promoter (CIITA-PI, -PII, -PIII, and -PIV) and is active in an overlapping subset of cell types [15]. CIITA-PIV is generally regarded as being responsible for IFNγ-inducible expression of CIITA [16,17], but it has also been described as being constitutively active in many non-hematopoietic cells [1,6,8,10,18]. In several instances, the silencing of CIITA-PIV promoter as well as its transitory inhibition have been held responsible for failure of IFNγ to induce MHCII transcription and downregulation of basal MHCII expression [19–26]. Moreover, a study on the effects of CIITA-PIV knockout in transgenic mice demonstrated that the selective deletion of CIITA-PIV does not seem to dramatically affect MHCII expression in professional APCs while has a significant

[1] These authors contributed equally to this work.
[2] Present address: Department of Internal Medicine and Medical Specialties, Università La Sapienza, Rome, Italy.
[*] Correspondence to: Division of Endocrinology, Department of Medicine, Columbia University Medical Center, 650W 168th street, New York, NY 10032, USA. Tel.: +1 212 305 7363; fax: +1 212 305 7348.
E-mail address: am86@columbia.edu (A. Maffei).

2211-2839
http://dx.doi.org/10.1016/j.rinim.2012.09.001

effect on MHCII expression in other APCs [27].

Interferon α (IFNα) is a type I IFN with an important role in the pathogenesis of several autoimmune diseases [28] and cancer immunotherapy [29]. In many cell types, type I IFNs block the induction of MHCII expression by IFNγ [30]. We recently demonstrated that the treatment with IFNα of human pancreatic islets *ex vivo* downregulates the CIITA-PIV-driven MHCII constitutive expression in non-professional APCs associated with islets [6]. In our system, the effect of IFNα-treatment on MHCII molecules was in contrast with the effect observed in professional APCs, where this cytokine upregulates the expression of MHCII genes. Other examples of discordance of IFNα-responsiveness in non-professional (melanoma cells) vs. professional APCs (immune cells) are described in human and mouse systems [31–33]. Apparently, similar to what happens with IFNγ, the biological effect of IFNα on MHCII expression is primarily mediated via the activation of the JAK/STAT pathway and the subsequent regulation of CIITA [30,34] by modulation of the promoter IV of this gene [6,35].

The aim of our study is to identify how the molecular system associated with the inhibitory function of IFNα on MHCII regulation in non-professional APCs is different from the system that mediates IFNα-induction of MHCII molecules in cells from the immune system (i.e., professional APCs). We believe that an understanding of these contrasting mechanisms can help in developing therapeutic strategies based on the tissue-specific regulation of MHCII gene expression in autoimmunity and transplantation.

The results presented in this paper provide experimental evidence supporting a simple mechanism that can account for the IFNα-mediated downregulation of MHCII in those non-professional APCs where the expression of these genes is mostly due to the constitutive activation of CIITA-PIV. We believe that this mechanism is due to the activation of the general negative feedback regulatory circuit of IFNα in the context of a constitutive weak expression of the target gene (CIITA-PIII and CIITA-PIV). On the basis of these results we formed the idea that it might be possible to mimic the IFNα-mediated downregulation of MHCII on these cells without the other (frequently unwanted) effects of this cytokine. To this purpose, we tested the effectiveness of using the RNA interference technology to selectively knock down the CIITA-PIV-driven expression of MHCII in non-professional APCs by specifically targeting CIITA-PIV mRNA.

2. Materials and methods

2.1. Reagents and cell lines

The Me10538 and M14 cell lines were both established from specimens obtained from primary tumors of melanoma patients [36,37]. The SK MEL-23 cell line was derived from a metastatic lesion of human melanoma [38]. The U87 cell line was derived from human malignant gliomas (ATCC HTB-14) [39]. All cell lines were cultured in RPMI Medium 1640 with 10% FCS (GIBCO) and 1% penicillin/streptomycin (Sigma). Recombinant human interferon gamma (IFNγ) was purchased from Peprotech, and recombinant human interferon alpha 2 b (IFNα) was purchased from PBL Biomedical Laboratories. Viability of cells after different treatments was measured through flow cytometry with 7-AAD and annexin V-FITC staining (BD Biosciences).

2.2. Flow cytometry analysis

Determination of cell surface expression of MHC class I (MHCI) and MHCII molecules was carried out by cytofluorimetric analysis using the FACS ARIA cell-sorting system and DIVA software (BD Biosciences). Direct immunofluorescence was executed using FITC mouse anti-human HLA-DR, -DQ and -ABC antibodies, along with the appropriate FITC mouse IgG isotype controls, all purchased from BD Biosciences. Staining, washing and analysis were performed as per the manufacturer's recommendations.

2.3. Measurement of specific transcripts by quantitative RT-PCR

Total RNA from cells was isolated using the RNeasy Mini Kit from QIAGEN. All Reverse Transcription reactions were performed using the QuantiTect RT Kit (QIAGEN). The accumulation of specific transcripts was measured by real-time PCR using the DNA Engine Opticon Real-Time PCR Detection System (BIORAD). The qPCR assays were performed using the quantity of cDNA obtained by reverse transcribing 10 ng of total RNA. The QuantiTect SYBR Green PCR Kit (QIAGEN) was used to perform all the reactions in the presence of 0.2 μM primers in a total volume of 25 μl. All primers used for qRT-PCR were synthesized by PRIMM, and their sequence and annealing temperature are presented in Table 1. Quantitative RT-PCR (qRT-PCR) reagent controls (reagents without any template or with 10 ng of not-reverse-transcribed RNA) were included in all the assays. Each assay was run in triplicate and the mean copy number from the three samples was used as the result of the single assay. Each assay was independently repeated at least three times and the mean copy number from the three assays was showed as the result of the experiment ± the standard error of the mean (SEM). The relative amount of specific transcripts was calculated by the comparative cycle threshold method presented by Livak and Schmittgen [40]. To correct for sample-to-sample variations in qRT-PCR efficiency and errors in sample quantitation, the level of GAPDH transcript was measured to normalize specific RNA levels. External standards were used to establish standard PCR curves for quantifying copies of transcripts that required an absolute, comparative quantitation. Fold-changes in expression were determined by dividing the normalized quantity of the gene of interest from IFNα-treated or IFNγ-treated cells by the normalized quantity of the gene of interest from untreated cells.

2.4. Western blot analysis

Total levels of STAT1, STAT2, P-STAT1, and P-STAT2 molecules were measured by immunoblot in protein extracts from IFN-treated and untreated cells. Antibodies specific for STAT1 (C-terminus), P-STAT1 (pY701), STAT2, P-STAT2 (pY690), were purchased from BD Biosciences, while the anti-mouse IgG (Fc specific)-peroxidase secondary antibody and the monoclonal anti-alpha-tubulin were from Sigma-Aldrich. Lysates were prepared from cells plated at 5×10^5 cells /well in 6-well plates with 2 ml of medium. Adherent cells were removed by brief treatment with trypsin and EDTA (Sigma-Aldrich) and then combined with non-adherent cells from the same culture and washed in cold PBS prior to being resuspended in 100 μl of RIPA buffer (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 1% NP40). Protease inhibitor cocktail tablets from Roche were added at 1 × concentration immediately prior to sample preparation. After 15 min of incubation at 4 °C with agitation, samples were centrifuged for 1 h at 4 °C and 12,500 rpm, and the recovered supernatant was divided into aliquots and stored at −80 °C until it was subjected to polyacrylamide gel electrophoresis. Protein concentrations were determined using a Bio-Rad protein assay (Bio-Rad Inc.) with bovine serum albumin standards, following the manufacturer's recommendations. Equal amounts of solubilized proteins (30 μg) were diluted in Laemmli sample buffer and subjected to electrophoresis on 12.5% acrylamide/bis gels. Proteins were then transferred onto PVDF membranes (Immobilon-P from Millipore) using an electroblotting system from Biometra. Membranes were prepared for immunoblotting by washing in TTBS (10 mM Tris–glycine, pH 8.0, 0.15 M NaCl, with 0.05% (w/v) Tween-20). Membranes were then blocked in TTBS plus 5% (w/v) non-fat dry milk for 1 h, followed by three 5 min washes in TTBS. Membranes were probed for specific proteins by 1 h incubations with the specific antibodies at the dilution suggested by the manufacturers. The membranes were then washed three times in TTBS and developed with the recommended dilution of the secondary antibody. After 1 h, the membranes were washed in TTBS, and the proteins on