Cellular Immunology 275 (2012) 80–89

Contents lists available at SciVerse ScienceDirect

Cellular Immunology

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

Glucocorticoid receptor mediated suppression of natural killer cell activity: Identification of associated deacetylase and corepressor molecules

Kristin A. Bush [a], Karen Krukowski [a], Justin L. Eddy [a], Linda Witek Janusek [b], Herbert L. Mathews [a,*]

[a] Department of Microbiology and Immunology, Stritch School of Medicine, Loyola University of Chicago, Maywood, IL 60153, USA
[b] Marcella Niehoff School of Nursing, Loyola University of Chicago, Maywood, IL 60153, USA

ARTICLE INFO

Article history:
Received 21 October 2011
Accepted 10 February 2012
Available online 21 March 2012

Keywords:
Natural killer cell
Glucocorticoid receptor
Epigenetic
Histone deacetylase
Corepressor

ABSTRACT

Physical and psychological stressors reduce natural killer cell function. This reduction in cellular function results from stress-induced release of glucocorticoids. Glucocorticoids act upon natural killer cells to deacetylate and transrepress immune response genes through epigenetic processes. However, other than the glucocorticoid receptor, the proteins that participate in this process are not well described in natural killer cells. The purpose of this study was to identify the proteins associated with the glucocorticoid receptor that are likely epigenetic participants in this process. Treatment of natural killer cells with the synthetic glucocorticoid, dexamethasone, produced a significant time dependent reduction in natural killer cell activity as early as 8 h post treatment. This reduction in natural killer cell activity was preceded by nuclear localization of the glucocorticoid receptor with histone deacetylase 1 and the corepressor, SMRT. Other class I histone deacetylases were not associated with the glucocorticoid receptor nor was the corepressor NCoR. These results demonstrate histone deacetylase 1 and SMRT to associate with the ligand activated glucocorticoid receptor within the nuclei of natural killer cells and to be the likely participants in the histone deacetylation and transrepression that accompanies glucocorticoid mediated reductions in natural killer cell function.

1. Introduction

Stress negatively impacts immune function. For example, stress reduces natural killer (NK) cell activity [1–4] through the activation of the hypothalamic–pituitary–adrenocortical axis and the production of increased levels of circulating glucocorticoid [5]. Glucocorticoids (GCs) are known to reduce histone acetylation and to transrepress immune response genes, including those genes that mediate NK cell effector function [6–9]. GCs exert their effect by interaction with the glucocorticoid receptor (GR). GR is a ligand activated transcription factor, which is a member of the nuclear receptor super family of proteins. It predominantly exists within the cytoplasm, but when ligand activated, GR translocates to the nucleus [10]. GR's subcellular location is determined by the accessibility of GR's nuclear localization signals and nuclear retention signal [11]. GC:GR interactions in the nucleus affect histone acetylation status by both inhibiting the activity of histone acetyltransferases (HATs) and by recruiting histone deacetylases (HDACs) and corepressor complexes [12], resulting in decreased acetylation of histones, chromatin compaction, and reduced gene expression [6,9]. Chromatin compaction reduces the expression of molecules that mediate natural killer cell activity (NKCA), including perforin and granzyme B [8,9,13–15].

HDACs are enzymes that remove acetyl groups from the epsilon amino lysines of the N-terminal tails of histone proteins. In general, increased levels of histone acetylation are associated with increased transcription, while decreased acetylation is associated with transcriptional repression. HDACs are associated with large multiprotein complexes such as, mSin3A, nucleosome remodeling and histone deacetylation (NURD), corepressor for RE1 silencing transcription factor (CoREST), silencing mediator of retinoic acid and thyroid hormone (SMRT), and nuclear receptor corepressor (NCoR). HDACs are divided into four major classes, Class I–IV, based on their homology with yeast orthologs. Class I HDACs includes HDAC1, 2, 3, and 8 associate with the four distinct multiprotein complexes identified above and are ubiquitously expressed with their functional activity optimized when associated with nuclear corepressor complexes. In contrast to HDAC1, 2, 3, HDAC8's functional activity is limited to smooth muscle [15,16]. Class I HDACs are known to be involved in epigenetic regulation of lymphocyte function, as transcriptional repressors. The capacity of Class II–IV HDACs to repress transcription in lymphocytes is less well characterized and in many cases, the expression of Class II–IV is non-lymphoid. Both NCoR and SMRT are known to repress genes important for NK cell function and are also known to associate with GR [27].

* Corresponding author. Address: Department of Microbiology and Immunology, Loyola University of Chicago, 2160 South First Ave., Maywood, IL 60153, USA. Fax: +1 708 216 9574.
E-mail address: hmathew@lumc.edu (H.L. Mathews).

0008-8749/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellimm.2012.02.014

Class I HDACs are known to associate with both SMRT and NCoR [17–21] and both HDAC1 and HDAC2 can be recruited by GR which is not necessarily the case for the other classes of HDACs [22,23].

The immunosuppressive effects of GCs are well documented [5,24–26] and include the reduced production of immune effector molecules like perforin and granzyme B [9]. Diminished production is due to decreased transcription of the genes subsequent to decreased acetylation of lysine residues on histone tails [9]. Although decreased histone acetylation is observed during GC treatment, the molecular mechanism by which this occurs is unknown. It is known that histone deacetylase (HDAC) inhibitors restore histone acetylation status, transcript and protein levels of both effector molecules and cytokines, in GC treated NK cells [9]. Thus it is probable that GR recruits HDAC(s) that mediate histone deacetylation and associated with these HDAC(s) are corepressor(s) that mediate transrepression. Thus the aim of this study was to determine whether Class I HDAC(s) and/or corepressor complex(es) were associated with GR in the nuclei of NK cells.

2. Materials and methods

2.1. Cell culture

The human erythroleukemic-like cell line, K562, was obtained from the American Type Culture Collection. K562 cells were maintained in suspension culture in Corning 75 cm^2 tissue culture flasks (Corning Glass Works, Corning, NY) in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS) low LPS (Gibco Laboratories, Grand Island, NY), 100 units/ml penicillin, 100 μg/ml streptomycin (Whittaker M.A. Bioproducts, Walkersville, MD), 0.1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, and 2 mM L-glutamine (Gibco Laboratories, Grand Island, NY).

The natural killer-like YT-Indy cell line (established from a child with acute lymphoma and thymoma [28]) was obtained from Christopher J. Froelich, M.D., Northshore University, Evanston, IL. YT-Indy cells were cultured in media containing RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 12% fetal bovine serum (FBS) low LPS (Gibco Laboratories, Grand Island, NY), 100 units/ml penicillin (Invitrogen, Carlsbad, CA), 100 μg/ml streptomycin (Whittaker M.A. Bioproducts, Walkersville, MD), 0.1 mM non-essential amino acids, 0.1 mM 2-mercaptoethanol, and 2 mM L-glutamine (Gibco Laboratories, Grand Island, NY).

2.2. Cellular treatment

YT-Indy cells, cultured at 2.5×10^5 cells/ml, were treated in 75 cm^2 tissue culture flasks with 100 nM (10^{-7} M) dexamethasone (Dex) (Sigma–Aldrich, St. Louis, MO) for 2, 4, 8, 12, and 24 h. This concentration of Dex did not decrease cell viability and is a concentration demonstrated previously to differentially regulate Dex responsive genes [29]. This concentration of Dex approximates physiological concentrations [30,31]. After treatment with Dex, YT-Indy cells were washed and resuspended to 5×10^6 cells/ml with media lacking all supplements. Cells treated with Dex for 2, 4, and 8 h were used for subcellular localization assays. Cells treated with Dex for 4, 8, 12, and 24 h were used for NKCA. Cells treated with Dex for 4 h were used for co-immunoprecipitation analysis. Cells treated with Dex for 4, 8, and 24 h were used for histone analysis. Cells treated with Dex for 4 h were used for co-immunoprecipitation analysis. Cells were treated with RU-486 (Sigma–Aldrich, St. Louis, MO) for 24 h. Cell number and viability were determined by vital dye exclusion using 0.1% Trypan Blue.

2.3. Natural killer cell activity (NKCA)

YT-Indy cell lytic activity (NKCA) against tumor targets was assessed using a standard chromium release assay, as previously described [32]. K562 tumor target cells were radioactively labeled with 100 μCi of [^{51}Cr] (New England Nuclear, Boston, MA). Radiolabeled K562 cells were incubated for 3 h with YT-Indy cells. Following incubation, the supernatants were removed using a Skatron harvesting press and the associated radioactivity was determined. Effector to target ratios for NKCA were 30, 20, and 10:1.

Results are expressed as percent cytotoxicity, calculated as:

$$\%\ \text{Cytotoxicity} = \frac{[(\text{experimental DPM}^*) - (\text{minimum DPM})]}{[(\text{maximum DPM}) - (\text{minimum DPM})]} \times 100.$$

All experimental means were calculated from triplicate values. Lytic units (LU) were calculated using a program written by David Coggins, FCRC, Frederick, MD. LU represents the number of cells per 10^7 effectors required to achieve 20% lysis of the target cells. ∗DPM = disintegrations per minute.

2.4. Immunofluorescent flow cytometric analysis of intracellular perforin

After treatment as described above, YT-Indy cells (1×10^5/assessment) were fixed and permeabilized with Cytofix/Cytoperm solution (BD Pharmingen, San Jose, CA) for 20 min at 4 °C. The cells were then washed twice with Perm/Wash Buffer (BD Biosciences, San Jose, CA) and then probed with antibodies specific for perforin (BD Biosciences, San Jose, CA) for 1 h at 4 °C. Following antibody staining the cells were washed twice with Perm/Wash Buffer (BD Biosciences, San Jose, CA). After staining samples were analyzed by flow cytometry with a FACSCanto equipped with a 15 mW argon-ion laser and a red diode laser using FACSDiva software for data acquisition [33–35]. 10,000–30,000 events were recorded and analyzed with FlowJo v8.4.1. Flow cytometric analysis was confirmed by fluorescence microscopy.

2.5. Subcellular localization

Nuclear and cytoplasmic fractions of YT-Indy cells were separated from 5×10^6 cells via the Fermentas ProteoJET Cytoplasmic and Nuclear Protein protocol (Fermentas, Burlington, ON). Nuclei were lysed using non-denaturing lysis buffer (20 mM Tris HCl pH = 8, 137 mM NaCl, 10% glycerol, 1% Nonidet P-40, 2 mM EDTA). Both lysed nuclear and cytoplasmic fractions were resuspended in Laemmelis SDS-sample buffer (4×) (Boston Bioproducts, Boston, MA). Samples were boiled for 5 min and proteins were separated by electrophoresis with a 10% polyacrylamide gel and transferred to nitrocellulose membrane for immuno-blotting. Proteins were visualized with anti-GR alpha (AbCam, Cambridge, MA), anti-HDAC1 (Millipore, Temecula, CA), anti-HDAC2, anti-HDAC3 or anti-acetylated histone 4 antibodies (AbCam, Cambridge, MA), horseradish peroxidase (HRP) conjugated anti-IgG secondary antibody (Millipore, Temecula, CA), and chemiluminescence reagent (ThermoScientific, Rockford, IL). Nuclear and cytoplasmic separation efficiency was determined with anti-LaminB1 (AbCam, Cambridge, MA) anti-GAPDH (Cell Signaling, Danvers, MA) antibodies, respectively. LaminB1 served as the control for nuclear contents, as it is localized to the nucleus only; GAPDH is only found within the cytoplasm, and was used as a control for cytoplasmic contents (See Supplemental Fig. 1 for an example of the efficiency of separation of nuclear and cytoplasmic constituents.) Blot density was quantified using ImageJ software.