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Idiotypic T cells specific for Morbillivirus nucleocapsid protein process and present their TCR to cognate anti-idiotypic CD8$^+$ T cells

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Abstract

CD8$^+$ T cells are activated by the presentation of antigenic peptide through MHC class I molecules. Newly synthesized proteins formed as defective ribosomal products (DRiPs) can act as a major source of antigenic peptides for MHC class I presentation pathway. Majority of these peptides are generated from the intracellular degradation of self antigens. In the present study, we have shown that newly synthesized T cell receptor (TCR) beta chains formed as DRiPs in T cells are ubiquitinated and degraded by the proteasomes. These TCR-DRiPs are processed and presented by activated T cells to cognate anti-idiotypic CD8$^+$ T cells. Presentation of TCR idiopeptide (peptide derived from the variable region of idiotypic TCR) by activated T cells leads to Bcl-2 expression and cytokine secretion by anti-idiotypic CD8$^+$ T cells. Presentation of intracellular antigen by T cells may have important implications in immunoregulation, control of lymphotropic virus infection and autoimmune diseases.

Keywords: T-APC; Proteasome; DRiPs; Anti-idiotypic T cells

1. Introduction

T cell immune responses are initiated by T cell receptor (TCR) recognition of pMHCs on APCs [1]. In case of MHC class I molecules, peptides derived mostly from newly synthesized proteins are generated by the multicatalytic proteasome, and transported through TAP-complex [2,3]. These peptides can be subjected to further N-terminal trimming by as yet undefined peptidases resident in endoplasmic reticulum (ER) to generate the 8 and 9 residue peptides required by class I MHC molecules [4]. Proteasomes are abundant macromolecular assemblies present in the nucleus and cytosol and are used by all eukaryotic cells to degrade unwanted proteins, usually those that are damaged or misfolded. These include nuclear, cytosolic and proteins in ER, which are re-exported into cytosol for degradation by proteasomes [5]. The major means of tagging proteins for proteasomal destruction is covalent modification by multiple chains of ubiquitin (Ub) protein [6]. Proteasomes participate in the generation of many peptides that are displayed on the surface complexed with MHC class I molecules for surveillance by the immune system [4]. Peptides are generated indiscriminately from host gene products as well as gene products of viruses and intracellular pathogens [4]. It has been shown that during the process of protein synthesis, ∼30% newly synthesized proteins are formed as defective ribosomal products (DRiPs) [3]. These DRiPs act as a source of antigenic peptides to provide trigger for cognate T cells. Discrimination between self and non-self peptides is performed at the level of the T cell receptor expressed by CD8$^+$ T lymphocytes. There is sufficient plasticity in the system to enable T cell recognition of some self antigens, which accounts for the generation of well-defined T cell responses to tumors and autoimmune diseases. Apart from the endogenous antigen presentation to MHC class I molecules, exogenous antigen can also be presented by class I either through cross-priming or cross-presentation. These pathways have been demonstrated for bacterial, viral and tumor antigens [7–10].

Endogenous self or non-self antigen presentations by different cell types are well characterized as all nucleated cells express MHC class I molecules. Presentations of self antigen have been proposed to have deleterious effect and leads to generation of autoimmune diseases. These autoimmune diseases can be controlled by the immunization of anti-idiotypic T cells [11–13]. The variable region of the TCR peptide which gets recognized by the self reactive T cells has been proposed to control the

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self reactive population in T cell vaccination [11]. However, the pathways by which these anti-idiotypic T cells are generated are not well established. These idiotypic and anti-idiotypic T cell populations are known to be present in the healthy individual [14,15].

We have used rinderpest virus (RPV) nucleocapsid protein (N) as antigen for generating antigen-specific idiotypic T cell in mice and show that TCR of these cells are recognized by anti-idiotypic T cells. RPV is a lymphotropic virus belonging to morbillivirus genus under paramyxoviridae family and causes a devastating disease in large ruminants [16] The nucleocapsid protein is the most abundant protein in infected cells and induces strong T cell response in cattle as well as mice [16,17]. The immunodominant cytotoxic and helper T cell epitopes of this protein has been characterized [17].

In the present study, we demonstrate that during protein synthesis, DRiPs are formed in mouse T cells. The newly synthesized TCRs are part of these DRiPs, which are ubiquitinated and degraded by the proteasome. The N antigen-specific TCR-DRiPs are processed and presented by activated idiotypic T cells to cognate anti-idiotypic T cells. The presentation of idiopeptide (peptide derived from the variable region of idiotypic TCR) by activated T cells leads to Bcl-2 expression and cytokine secretion by anti-idiotypic $CD8^+$ T cells.

2. Materials and methods

2.1. Animals

Balb/c mice were bred and maintained in the Central Animal Facility, Indian Institute of Science, Bangalore, India. Six to eight weeks old male mice were used in all experiments following institutional ethical use of animal protocols.

2.2. Reagents

The following antibodies: purified anti-mouse I-A/I-E (clone M5/114), anti-mouse CD11c (clone HL3), anti-mouse IFN-γ-biotin (XMG1.2), purified recombinant mouse IL-2 and isotype control antibodies were purchased from PharMingen, USA. Anti-mouse TCRβ-Cy5PE (H57.597) and Streptavidin-Cy5PE were from e-Bioscience, USA. Donkey anti-mouse IgG-FITC and donkey anti-rabbit IgG-FITC were obtained from Jackson ImmunoResearch Laboratories, USA. Purified anti-mouse TCR α/β antibody (H57.597) is a generous gift from Dr. P. Marrack, HHMI, USA. Recombinant rinderpest virus nucleocapsid protein (N protein) and phosphoprotein (P protein) were expressed in *E. coli* and purified as described earlier [17,18].

2.3. Purification of T cells

T cells were purified as described earlier [19]. Briefly, mice were killed by cervical dislocation. Inguinal, posterior axillary, internal axillary and popliteal lymph nodes were removed and single cell suspensions were prepared. Cells were panned for 1 h in fetal bovine serum (FBS) (Gibco BRL, USA) coated plate (Falcon). Unbound cells were harvested and complement-mediated lysis was performed using anti-mouse Ig, anti-mouse MHC class II (clone m5/115), anti-mouse CD11c and rabbit complement. Mononuclear cells were purified by density gradient centrifugation on Histopaque®-1077 (Sigma, USA). Cells were further panned on anti-mouse Ig antibody coated plate for 1 h in complete medium (RPMI-1640 supplemented with 25 mM HEPES, 100 μg/ml penicillin, 100 μg/ml streptomycin, 20 μg/ml gentamicin and 10% FBS). Unbound cells were harvested and the purity of T cells was monitored by FACS and found to be 99% pure.

2.4. Radiolabeling and immunoprecipitation

Radiolabeling and immunoprecipitations were performed as described earlier [20]. Before radiolabeling, purified T cells were incubated for 1 h in cysteine-methionine-deficient RPMI-1640 (Sigma) supplemented with 5% dialyzed FBS and 200 unit IL-2. The cells were then pelleted and re-suspended in the same medium at 4×10^7 cells/ml and metabolically labeled by pulse labeling for 20 min with 200 μCi/ml [^{35}S] methionine protein labeling mix (Perkin-Elmer, USA). After the pulse, the cells were pelleted, unincorporated radiolabel removed by washing, and the cells were resuspended at 2×10^6 cells/ml in pre-warmed RPMI medium containing 5% FCS supplemented with 200 unit IL-2. Cells were then incubated for 0, 15, 30 or 60 min with or without lactacystin (5 mM) in complete RPMI medium at 37 °C in a 5% CO_2 atmosphere.

After the pulse–chase, the cells were pelleted and lysed in 0.5% Triton X-100, 300 mM NaC1, 50 mM Tris, pH 7.4, complete protease inhibitor cocktail (PharMingen, USA). Nuclei and debris were eliminated by centrifugation. The cell lysates were pre-cleared first, by an incubation with rabbit anti-mouse IgG (Sigma) and Protein-A agarose (Bangalore Genie, India) overnight at 4 °C. Lysates were incubated with anti-mouse TCR α/β antibody (H57.597) for 2 h. Immune complexes were precipitated using Protein-G agarose (Sigma). Aggregate immune-complexes were washed three times in 0.5% NP40, 30 mM NaC1, 50 mM Tris and pH 7.4. The beads were then re-suspended in Laemmli sample buffer and boiled for 3 min. The immunoprecipitates were analyzed by electrophoresis using 12% SDS-PAGE. The gels were treated with salicilate, and dried. The gels were exposed to phosphoimager plate for 16 h and scanned.

2.5. Trichloroacetic acid (TCA) precipitation

TCA precipitations performed as described earlier [3]. Briefly, aliquot of cell lysates were spotted onto Whatmann GF/C filters, washed with 10% (w/v) TCA and twice more with 70% ethanol then dried. The incorporated radioactivity was measured in a scintillation spectrometer.

2.6. Western blotting

Purified mouse T cells (4×10^6 cells/sample) were cultured in complete RPMI medium in presence or absence of lactacystin (5 mM) for 3 h. Cells were lysed in lysis buffer (50 mM