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Cross-presentation of a human malaria CTL epitope is conformation dependent

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Abstract

Little is known about the role of conformation on the antigen processing by antigen presenting cells. Using a well-defined antigen containing two disulfide bridges, the synthetic C-terminal fragment 282–383 derived from *Plasmodium falciparum* circumsporozoite protein (*Pf*CS 282–383), we show that the reduced form is presented in vitro more efficiently than its oxidized counterpart, inducing stronger CTL recognition. In addition, only the reduced form can be presented by the TAP independent T2 cell line. Thus, the reduced form is processed by TAP dependent and independent pathways.

Keywords: Antigen presentation; 3D structure; Malaria; Circumsporozoite protein

1. Introduction

Classical MHC class I molecules bind peptides usually generated from endogenously processed proteins. In this process, cytosolic proteins are hydrolyzed by the proteasome complex to yield peptides that are translocated into the endoplasmic reticulum (ER) through the transporter associated with antigen presentation (TAP). In the ER, the peptides bind MHC class I molecules and are then transported to the cell surface for recognition by $CD8^+$ T cells (Pamer and Cresswell, 1998).

In the case of proteins that are not synthesized intracellularly, a way to introduce exogenous peptides into the "classical" MHC class I pathway and activate CTL is through a process called cross-presentation, a mechanism originally described by Bevan (1976) and in which dendritic cells (DC) are the most efficient antigen presenting cells (APCs) (Wilson and Villadangos, 2005). Three different models were proposed to explain the cross-presentation pathway (Heath et al., 2004). In the first model, referred to as the vacuolar pathway or TAP independent pathway, antigenic peptides are generated by proteolytic enzymes inside a compartment that is comprised of the fusion between a phagosome and a ER-derived vesicle. In the second TAP dependent model, the phagosomes fuse with the ER membrane, a process which enables the transfer of the antigen presentation machinery from the ER to the phagosome, which therefore act as organelles competent for cross-presentation (Guermonprez et al., 2003; Houde et al., 2003). Other studies have also reported the translocation of exogenous antigens, from the endosomal compartment directly into the cytosol, and therefore enabling the antigen to be shifted into the classical MHC class I pathway (Brossart and Bevan, 1997; Moron et al., 2003; Prato et al., 2005). A third model is represented by retrograde pathway in which soluble molecules reach the perinuclear ER (Ackerman et al., 2005). In addition, it has been shown that extracellular proteins can also gain access to the MHC class I pathway using other pathways in which cell surface or serum proteases also have the capability to process extracellular antigens and generate MHC class I-restricted peptides that can bind molecules on the cell surface directly (Yewdell et al., 1999; Thai et al., 2004).

In a recent in vitro study (Prato et al., 2005), we showed that monocyte-derived dendritic cells (Mo-DC) and EBV-transformed B-lymphoblastoid cells (LCL) were able to cross-

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present the oxidized form of the 102-mer polypeptide, representing the C-terminal region (amino acid 282–383) of the CS protein of *Plasmodium falciparum* sporozoite, known as *Pf*CS 282–383 (Roggero et al., 1995). We showed that the presentation of the CTL epitope (*Pf*CS 327–335) to BF7-67 cells, by both APCs, followed an endosome-to-cytosol pathway that involved the proteasome and the "classical" MHC class I pathway. Furthermore, in Mo-DC, the presentation of the polypeptide required additional processing by endosomal/lysosomal proteases. Surprisingly, the strength of the response elicited by both APC was comparable. While it has been previously shown that LCL and non-professional APC such as TAP-deficient T2 cells can cross-present soluble proteins (Gnjatic et al., 2003), our finding that *Pf*CS 282–383 is efficiently presented by cells other than DC are of particular biological relevance. The cell implicated in cross-presentation of sporozoite antigens during malaria infection would be the hepatocyte, a cell without specialized antigen processing capability.

Very few reports have studied the dependency of T cell activation on antigen structure (Buchmuller and Corradin, 1982; Griem et al., 1996; Michalek et al., 1996; Thai et al., 2004; Hensmann et al., 2004). Hence, here, we investigated whether a change in the conformation (oxidized form versus reduced form) of the synthetic *Pf*CS 282–383 polypeptide had an effect on the antigen processing and presentation by LCL and the TAP-deficient T2 cell.

2. Materials and methods

2.1. Peptides

The synthetic *Pf*CS 282–383 polypeptide represents the C-terminal region (amino acids 282–383) of the CS protein of *P. falciparum* strain NF54 (Roggero et al., 1995) synthesized as previously described with some modification (Roggero et al., 1997). In particular, a His tail was not added at the N-terminus of the peptide for purification purposes, rather peptide was purified to homogeneity on a reverse phase C-18 HPLC column using a linear gradient of 0.1% TFA in water and acetonitrile (purity was determined by analytical HPLC and mass spectrometry; >90%). Folding of the polypeptide was performed by oxidation in air of the 4 cysteines, as determined by *N*-ethyl-maleimide reaction and mass spectrometry. The synthetic peptide representing the HLA-A*0201 binding sequence of *P. falciparum* strain NF54, *Pf*CS 327–335, was also produced using F-moc chemistry and purity was higher than 90%.

When indicated, the reduced polypeptide and the short peptide were incubated in 20 mM dithiothreitol (DTT) (Astral Scientific, NSW, Australia) for 24 h at 37 °C prior to cell pulsing (final DTT concentration during pulsing, 1 mM).

2.2. Cells

HLA-A*0201 LCL and TAP-deficient T2 cells (Hosken and Bevan, 1990) were maintained in RPMI 1640, 10% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine (all from Gibco Life Technologies).

*Pf*CS 327–335-specific $CD8^+$ CTL BF7-67 cells were generated and maintained as previously described (Bonelo et al., 2000).

2.3. Antigen pulsing assay

APCs were pulsed with various concentrations of oxidized or reduced *Pf*CS 282–383 polypeptide for 2 h at 37 °C in serum-free X-VIVO 15 medium (Biowhittaker, Walkersville, MD). As control, the cells were pulsed with or without 1 μM *Pf*CS 327–335 peptide during the same time at 37 °C.

2.4. Inhibition studies

APC (5×10^5 cells/well) were pre-incubated for 2 h with the various reagents prior to the addition of antigen and the same concentration of inhibitors was maintained during antigen pulse. The reagents used were: ammonium chloride (NH_4Cl, 10 mM), *N*-acetyl-L-leucyl-L-leucyl-L-norleucinal (LLnL, 50 μM), brefeldin A (BFA, 35 μM), cycloheximide (CHX, 35 μM), Amiloride (0.2 mM) (all from Sigma–Aldrich). After peptide pulsing, APC were washed twice, (×40 and ×1000 volumes), with RPMI 1640 medium and co-cultured with BF7-67 cells in absence of inhibitor.

2.5. Measurement of IFN-γ cytokine in culture supernatant

BF7-67 cells (1×10^4/well) were co-cultured with peptide-pulsed APC (1.5×10^5/well), for 20 h in 96-well plate containing 200 μl complete medium {CM; RPMI 1640, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), HEPES (10 mM), pyruvate (1 mM), non-essential amino acids (0.1 mM), 2-ME (0.05 mM)} (all purchased from Gibco Life Technologies). Supernatants were collected from each well and assayed for human IFN-γ content by ELISA (Mabtech Australia Pty. Ltd., NSW, Australia) according to the manufacturer's instructions.

2.6. Intracellular staining and flow cytometry analysis

For intracellular staining, BF7-67 cells (2×10^4 cells/well) were co-cultured in 96-well plate with peptide-pulsed APC (3×10^5 cells/well) for 4 h at 37 °C in 200 μl of CM plus 5% AB human pooled serum (Australian Red Cross Blood Service, ARCB, Brisbane) and the intracellular transport inhibitor BFA (10 μg/ml). After two washes, cells were stained with FITC-conjugated CD8 (SK1 clone, IgG1) (BD Biosciences, San Diego, CA) and 7-amino-actinomycin-D (7-AAD) (Sigma–Aldrich) at 4 °C for 20 min. Cells were washed three times and fixed for 10 min at room temperature. For measuring intracellular IFN-γ production, cells were washed three times and stained overnight at 4 °C in presence of PE-conjugated anti human IFN-γ (25723.11 clone, IgG2b) (BD Biosciences) and 0.2% saponin (Sigma–Aldrich). Analysis gated on viable lymphocytes population ($CD8^+$ $7\text{-}AAD^-$) was performed on a FACSCalibur (BD Biosciences) and Cellquest Pro software (BD Biosciences).