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IFN-α boosts epitope cross-presentation by dendritic cells *via* modulation of proteasome activity

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ABSTRACT

We have investigated the molecular mechanisms underlying the peculiar cross-presentation efficiency of human dendritic cells (DCs) differentiated from monocytes in the presence of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interferon (IFN)-α (IFN-DCs). To this end, we evaluated the capability of IFN-DCs to present and cross-present epitopes derived from Epstein–Barr Virus (EBV) or human melanoma-associated antigens after exposure to cell lysates or apoptotic cells. In an autologous setting, IFN-DCs loaded with Lymphoblastoid Cell Lines (LCL) lysates or apoptotic LCL were highly efficient in expanding, respectively, EBV-specific class II- or class I-restricted memory T cell responses. Of note, IFN-DCs loaded with apoptotic LCL were more potent than fully mature DCs in triggering the cytotoxicity of $CD8^+$ T lymphocytes recognizing a subdominant HLA-A*0201-restricted epitope derived from EBV latent membrane protein 2 (LMP2). In addition, IFN-DCs loaded with apoptotic human melanoma cells were highly efficient in cross-presenting the MART-$1_{27–35}$ epitope to a specific $CD8^+$ cytotoxic T cell clone, and this functional activity was proteasome-dependent. These IFN-DC properties were associated with an enhanced expression of all the immunoproteasome subunits as well as of TAP-1, TAP-2 and tapasin, and, interestingly, to a higher proteasome proteolytic activity as compared to immature or mature DCs. Altogether, these results highlight new mechanisms by which IFN-α influences antigen processing and cross-presentation ability of monocyte-derived DCs, with potentially important implications for the development of DC-based therapeutic vaccines.

Introduction

The term cross-presentation defines the process by which professional antigen-presenting cells (APCs) present peptides of antigens acquired from the extracellular environment *via* their own MHC class I molecules to $CD8^+$ T cells. It is now widely accepted that cross-presentation likely represents the most important mechanism for the priming of $CD8^+$ T cell responses against exogenous antigens *in vitro* and *in vivo* and that cross-presentation of antigens acquired from tumour and other donor cells is a very efficient process (Bevan 2006).

Whereas different APCs, such as macrophages, B cells, dendritic cells (DCs), endothelial cells and neutrophils, have all been shown to mediate antigen cross-presentation (Basta and Alatery 2007), and mouse neutrophils have been recently reported to cross-prime $CD8^+$ T cells *in vivo* (Beauvillain et al. 2007), DCs appear to possess a unique ability to cross-present exogenous antigens and to prime $CD8^+$ T cells to endogenous and exogenous antigens *in vivo* (Kurts et al. 2001; Probst and Van den Broek 2005; Jung et al. 2002). However, antigen cross-presentation does not necessarily result in the induction of an immune response, i.e. cross-priming, and can also be involved in the maintenance of tolerance to self-antigens (cross-tolerance) (Basta and Alatery 2007).

DCs can exist in two functional states, immature and mature, with mature DCs having the ability to prime an immune response (Steinman 1991). However, recent observations indicate that phenotypically mature DCs do not always promote T-cell immunity but may also induce tolerance (Reis e Sousa 2006). Thus, the characterization of the specific functions of phenotypically mature DCs is essential for understanding whether these DCs are immunogenic or tolerogenic (Reis e Sousa 2006).

Immature DCs (iDCs) are characterized by an efficient phagocytic activity that allows antigen up-take and processing (Banchereau and Steinman 1998). Likewise, iDCs tend to maintain peripheral tolerance to self-antigens *in vivo* by multi-

Abbreviations: HLA, human leukocyte antigen; LPS, lipopolysaccharide; STAT, signal transducers and activator of transcription; TAP, transporter associated with antigen processing; Th, T helper.

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ple mechanisms, including induction/stimulation of regulatory T cell populations (Cools et al. 2007). It has been reported that only mature DCs are efficient APCs for *in vitro* cross-priming of $CD8^+$ T cells against exogenous antigens (Reis e Sousa 2006; Banchereau and Steinman 1998; Schuler et al. 2003; Kaiser et al. 2003). During the maturation process, DCs become less efficient in antigen capturing but more specialized in processing and presenting immunogenic peptides and in activating naïve T cells, after migration to the lymph node (Banchereau and Steinman 1998). Maturation of DCs can be mediated by inflammatory cytokines, or by additional stimuli such as CD40L, LPS or virus infection. All these stimuli can trigger the up-regulation of MHC class I antigen-processing machinery as well as of costimulatory molecules (CD40, CD80, CD86) and of the DC maturation marker CD83 (Basta and Alatery 2007; Banchereau and Steinman 1998) necessary for T-cell activation.

In order to become competent to induce cross-priming, DCs need to receive an activation signal, a process called "licensing" of APCs (Banchereau and Steinman 1998). Cross-presentation of antigens by "not licensed" DCs stimulates an abortive response that culminates in cross-tolerance (Cools et al. 2007). A body of evidence indicates that the difference between tolerance and immunity depends on the presence or absence of inflammatory signals associated with viral or bacterial pathogens, as well as on costimulatory signals. Pathogen-associated molecular patterns (PAMP), such as lipopolysaccharide, double-stranded RNA, and unmethylated CpG oligodeoxynucleotides, are recognized by specific receptors, including the Toll-like receptors. Through their receptors, PAMPs activate DCs and promote immune responses (Creagh et al. 2006). In addition, soluble mediators released during infections can stimulate immune responses to cross-presented antigens (Beignon et al. 2003).

Among host-derived infection-associated signals that stimulate cross-priming are type I IFNs (IFN-α/β). These cytokines are expressed rapidly by cells in response to viral infection and play a crucial role in linking innate and adaptive immunity (Le Bon and Tough 2002). In particular, type I IFNs have been demonstrated to efficiently promote *in vivo* the cross-priming of $CD8^+$ T cells in mouse models (Le Bon et al. 2003) by acting on DCs (Le Bon et al. 2001).

Priming of $CD8^+$ T cells requires recognition through the T cell receptor of MHC class I-restricted peptides. These peptides are produced by the proteasome, a multicatalytic protease complex that degrades protein into peptides from 3 to 20 aminoacids in length. The active form of the proteasome is the 26S complex which recognizes and binds ubiquitinated proteins and is composed of a catalytic 20S core particle and a 19S regulatory particle. In all eukaryotic cells, the 26S proteasome complex is assembled in a four rings structure, with two outer rings consisting of seven non catalytic α type subunits and two inner rings composed of seven β type subunits (Murata et al. 2009). Three of these constitutively expressed β subunits possess catalytic activities and, upon stimulation of cells with IFN-γ, are replaced by inducible subunits called LMP2 ($i\beta1$), MECL1 ($i\beta2$), and LMP7 ($i\beta5$) that exhibit post-acidic, tryptic-like and chymotryptic-like activity, respectively (Murata et al. 2009), and constitute the so called immunoproteasome.

The immunoproteasome exerts an increased capability to cleave after hydrophobic and basic residues, which are the most frequent residues found at the COOH terminus of the MHC class I binding peptides (Van den Eynde and Morel 2001).

It is known that during DC maturation the proteasome regulator PA28α/β and the proteins involved in antigen transport and presentation such as TAP1, TAP2 and tapasin are up-regulated (Macagno et al. 1999, 2001; Gil-Torregrosa et al. 2004). It has been shown that immature DCs express similar levels of proteasomes and immunoproteasomes (Macagno et al. 1999). During maturation, the synthesis of immunoproteasomes is stimulated, but this increased synthesis does not seem to translate into an increased content of immunoproteasomes (Macagno et al. 2001). Moreover, it has been previously demonstrated that during mouse DC maturation the increase in proteasome activity is selective for the LMP2 and MECL1 catalytic subunits (Gil-Torregrosa et al. 2004).

Previous studies from our as well as other groups demonstrated that IFN-DCs in addition to be more efficient than immature IL-4-DCs in inducing a Th-1 type immune response and antigen-specific $CD8^+$ T cell responses (Santini et al. 2000; Lapenta et al. 2003, 2006; Santodonato et al. 2003; Gabriele et al. 2004; Tosi et al. 2004), have a special attitude for cross-priming $CD8^+$ T cells against viral antigens *in vitro* and *in vivo* (Lapenta et al. 2006). Whereas the superior cross-priming ability of IFN-DCs does not appear to result from an increased antigen up-take and endosomal processing activity, it might be attributed to a higher level of costimulatory molecules and HLA class I molecules expression as compared to mature IL-4-DCs or, alternatively, by the higher efficiency of IFN-DCs in targeting antigens onto class I processing pathway with respect to mature IL-4-DC counterparts (Lapenta et al. 2006). However, the molecular mechanisms and pathways underlying the peculiar efficiency of IFN-DCs in mediating antigen cross-presentation and cross-priming remained largely elusive. The present study aimed to analyze the possible differences between IFN-DCs and immature or mature IL-4-DCs in the molecular pathways of exogenous antigen processing and presentation. To this end, we chose the model system of cross-presentation of EBV- or melanoma-associated antigens. First, we evaluated the cross-presentation efficiency of IFN-DCs as compared to immature IL-4-DCs in a completely autologous setting, in which DCs from EBV-positive donors were loaded with apoptotic cells (apo-LCL) or cell lysates (lys-LCL) derived from autologous LCL, and then used as APC for the stimulation of autologous PBMCs. In parallel, we analyzed the expression levels of molecules involved in the cellular antigen processing and presentation machinery. In particular, we analyzed the expression levels and enzymatic activity of the proteasome complex. Then, we assessed the efficiency of IFN-DCs *vs.* immature or mature IL4-DCs in inducing, after loading with apoptotic LCL or human melanoma cells, (i) the cytotoxic activity of cytotoxic T lymphocytes (CTLs) specific for a sub-dominant $CD8^+$ T-cell epitope of the EBV LMP2 antigen (Gavioli et al. 2002), and (ii) the cross-presentation of the melanoma-associated immunodominant Melan-A/MART-$1_{27–35}$ epitope (Romero et al. 2006) to a specific $CD8^+$ T cell clone, in the absence or in the presence of the proteasome inhibitor lactacystin (Lee and Goldberg 1998).

Materials and methods

Cell lines

Lymphoblastoid Cell Lines (LCL) were established by *in vitro* infection of B lymphocytes from healthy donors typed positive for HLA-A2 or HLA-A11, A28 with B95.8 strain of EBV. The Juso HLA-A2-negative melanoma cell line (kindly provided by Giulio C. Spagnoli, Department of Biomedicine, University of Basel, Switzerland) and the LCL were cultured in RPMI-1640 supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated fetal calf serum (HyClone, Euroclone).

The T2 TAP-deficient HLA-A2-positive cell line was cultured in IMDM (Euroclone) supplemented with 10% FCS, 10^{-5} M 2-ME, L-glutamine, penicillin/streptomycin, sodium pyruvate, nonessential amino acids, and HEPES (Euroclone).