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Regulatory role of tryptophan degradation pathway in HLA-G expression by human monocyte-derived dendritic cells

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Abstract

Dendritic cells (DC) are strong inducers of immunity but they can also be tolerogenic. During monocyte differentiation to DC the immunosuppressive indoleamine-2,3-dioxygenase (IDO) is induced. IDO degrades Trp to kynurenine, which is further metabolized to 3-hydroxyanthranilic acid. DC can also express mRNA and protein of the tolerogenic molecule HLA-G, but there is no surface expression. We studied the effect of the Trp degrading pathway on HLA-G expression by DC. When monocytes were differentiated to immature DC in presence of either Trp or its metabolites kynurenine or 3-hydroxyanthranilic acid they expressed cell surface HLA-G, and Trp also increased shedding of HLA-G1. Trp induced HLA-G cell surface expression when present during maturation with IFN-γ + LPS, but not with TNF-α. Kynurenine increased HLA-G expression in both TNF-α and IFN-γ + LPS matured DC, and 3-hydroxyanthranilic acid had a very weak effect on HLA-G cell surface expression when present during maturation. Shedding of HLA-G1 was more pronounced in IFN-γ + LPS-matured DC than in immatured DC. Maturation with IFN-γ + LPS in presence of kynurenine also increased HLA-G5 secretion. The mechanism involved seems to be post-translational as mRNA and cellular HLA-G protein content was not increased with Trp, kynurenine or 3-hydroxyanthranilic acid treatments. Finally, immature DC preincubated with Trp, kynurenine and 3-hydroxyanthranilic acid have after a decreased capacity to stimulate T cells in mixed lymphocyte reaction. In IFN-γ + LPS-matured DC this decreased capacity was obtained with kynurenine and 3-hydroxyanthranilic acid. These results suggest that IDO can induce HLA-G cell surface expression in DC, and that these two molecules can cooperate in the immune suppression.

Keywords: Suppression; Tryptophan; Kynurenine; 3-Hidroxyanthranilic; Monocytes; Dendritic cells; HLA-G; Indoleamine-2,3-dioxygenase

1. Introduction

Dendritic cells (DC) play a central role in innate and adaptive immunity, and are strong activators of T cells (Grohmann et al., 2002). These cells exist as distinct subsets that differ in their ontogeny, surface molecule expression, and biological function. DC in peripheral tissue are resting, and endocytosis in absence of additional stimuli induces T-cell deletion and unresponsiveness (Hawiger et al., 2001). Monocyte-derived DC generated in vitro from peripheral blood with GM-CSF + IL-4 can be considered as immature DC which do not induce energy (Lutz and Schuler, 2002), while mature DC produced after stimulation can induce T-cell response in vivo (Banchereau and Steinman, 1998). However, it has been shown that the same DC can initiate a tolerance or immune activity, depending on the maturation, activation stage, and other factors (Lutz and Schuler, 2002; Steinman et al., 2003). These cells not only activate lymphocytes, but also tolerize T cells to self-antigens, thereby minimizing autoimmune reactions (Steinman et al., 2003).

The cytosolic heme protein indoleamine-2,3-dioxygenase (IDO) is the rate-limiting enzyme in the catabolism of tryptophan (Takikawa et al., 1988). IDO can be expressed by macrophages, monocytes and DC (Mellor and Munn, 1999). IDO catalyzes the oxygenative cleavage of the indole ring of various indoleamine derivatives, with wide sub-

Abbreviations: DC, dendritic cells; HLA-G, human leukocyte antigen G; IDO, indoleamine 2,3 dioxygenase; LPS, lipopolysaccharide; 1MT, 1-methyl-tryptophan; PBMC, peripheral blood mononuclear cells; sHLA-G1, shed HLA-G1

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strate specificity. Tryptophan catabolism via the IDO pathway yields *N*-formylkynurenine and kynurenine, which in human macrophages is further metabolized to 3-hydroxyanthranilic acid (Werner-Felmayer et al., 1989). IDO induction in monocytes and macrophages is part of the immune defense against bacteria (Carlin et al., 1989) and tumors (Ozaki et al., 1988). Recently, Munn and Mellor (2004) have demonstrated its role in immune tolerance, and its participation in the maternal tolerance to fetus in murine models (Munn et al., 1998). IDO is also used by cancer cells for immune escape. Different types of experiments have been performed to explain the inhibitory effect of IDO (Moffett and Namboodiri, 2003). Some of them have demonstrated that tryptophan breakdown suppresses T-cell proliferation by exhausting this critical amino acid from the medium (Munn et al., 1998). Other metabolites of the Trp degradation pathway, mostly kynurenine and 3-hydroxyanthranilic acid, also take up an active role in restraining lymphocytic response by inducing apoptosis of activated T cells (Fallarino et al., 2002; Terness et al., 2002).

HLA-G is a non-classical Class I human leukocyte antigen (HLA) which exists as four membrane-bound and three soluble isoforms (HLA-G1 to -G4, and HLA-G5 to -G7, respectively), that are generated by alternative splicing of a single primary transcript (Kirszenbaum et al., 1994; Moreau et al., 1995). Although it is expressed as a membrane-bound molecule, HLA-G1 can be shed (sHLA-G1) or proteolytically cleaved from the cell surface (Rebmann et al., 2005). HLA-G can bind three inhibitory receptors, ILT2, ILT4 and KIR2DL4. It has been demonstrated that both membrane-bound and soluble HLA-G isoforms can inhibit natural killer (NK) cell cytotoxicity, allogeneic proliferation of T cells, and antigen-specific T cell cytotoxicity (Carosella et al., 2003). The expression of HLA-G is normally restricted to thymic epithelial cells (Crisa et al., 1997), cornea (Le Discorde et al., 2003) and extravillous cytotrophoblast (Yang et al., 1995). HLA-G is expressed as early as the blastocyst stage and is crucial for implantation and early fetal-maternal tolerance (Carosella et al., 2003; Rouas-Freiss et al., 1997a). Even though HLA-G is not normally expressed in DC (Onno et al., 2000), it can be expressed in some pathological circumstances, such as DC infiltrating tumors, and non-tumoral diseases (Onno et al., 2000; Pangault et al., 2002).

HLA-G and IDO share some properties: they have tolerogenic capacity, they are highly expressed mainly in human placenta (Honig et al., 2004; Rouas-Freiss et al., 1997b), and can be expressed ectopically by tumors (Rouas-Freiss et al., 2003; Uyttenhove et al., 2003). Furthermore, their expression can be regulated by the same cytokines, i.e. IFN-γ (Takikawa et al., 1988; Yang et al., 1996) and IL-10 (Moreau et al., 1999; Munn et al., 2002). We have previously shown that IDO blockade can induce HLA-G cell surface expression in monocytes, while Trp down-regulates its surface expression (Gonzalez-Hernandez et al., 2005), and that HLA-G and IDO have independent tolerogenic pathways (Le Rond et al., 2005). IDO is one of the genes highly transcribed in mature DC (Dietz et al., 2000), and also monocyte-derived DC express HLA-G, but not the surface protein (Le Friec et al., 2004). For this reason, the aim of this work was to know the relationship between Trp catabolism and HLA-G in DC. Results shown here demonstrate that Trp, and the metabolites kynurenine and 3-hydroxyanthranilic acid can induce HLA-G in immature and mature DC. Also these compounds provoked a decreased stimulatory capacity of DC.

2. Methods

2.1. Cell culture

Culture medium throughout the study was RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum, 50 U/mL penicillin, 50 μg/mL streptomycin and 2 mM L-glutamine (Gibco, CA, USA). Cells were cultured at 37 °C in a 5% CO_2 humidified atmosphere.

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized whole blood of normal healthy volunteers by standard density gradient centrifugation with Ficoll-Paque (Pharmacia, Uppsala, Sweden). Monocytes were differentiated to dendritic cells with GM-CSF + IL-4 and matured with TNF-α or IFN-α + LPS according to the methodology already described (Fernandez-Ruiz et al., 2004; Pickl et al., 1996). Trp, kynurenine or 3-hydroxyanthranilic acid (Sigma–Aldrich, MO, USA) was added with cytokines during differentiation or maturation. We used a lower 3-hydroxyanthranilic acid concentration compared to Trp and kynurenine to avoid the toxic effect of this compound (Fallarino et al., 2002). Cell viability checked by trypan blue exclusion method was greater than 95%, and apoptosis measured by the annexin V method using a commercial kit (Immunotech, France) was lower than 10%.

Human choriocarcinoma cell line JEG-3 was obtained from the American Type Culture Collection and cultured using DMEM medium according to provider instructions.

2.2. Indoleamine 2,3-dioxygenase activity assay

IDO activity was evaluated by measuring kynurenine in the culture medium according to Alegre et al. (2005). Briefly, culture mediums were boiled for 10 min and centrifuged at 2500 × *g* for 10 min. Kynurenine in the supernatant was measured in a HPLC system with a Waters C18 column (4.5 mm × 15 cm). The mobile phase was 15 mM acetic acid–sodium acetate (pH 4.0) containing 27 mL/L acetonitrile, and kynurenine was detected by absorbance at 360 nm.

2.3. Mixed lymphocyte reaction

Responder PBMC were plated into 96-well round-bottomed microtitre plates at 200,000 cells/wells together with mismatched immature and mature DC as stimulators cells. The ration DC:PBMC was 1:10, 1:20 and 1:40 and all samples were run in triplicate. Cultures were pulsed after four days of MLR with tritiated thymidine (1 μCi per well, Amersham Biosciences, Uppsala, Sweden). Eighteen hours later, cells were harvested on filter mats and thymidine incorporation was quantified on a β-counter (Wallac 1450, Amersham Biosciences).