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Ecto-F_1-ATPase and MHC-class I close association on cell membranes

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Abstract

Subunits of the mitochondrial ATP synthase complex are expressed on the surface of tumors, bind the TCR of human Vγ9/Vδ2 lymphocytes and promote their cytotoxicity. Present experiments show that detection of the complex (called ecto-F_1-ATPase) at the cell surface by immunofluorescence correlates with low MHC-class I antigen expression. Strikingly, the α and β chains of ecto-F_1-ATPase are detected in membrane protein precipitates from immunofluorescence-negative cells, suggesting that ATPase epitopes are masked. Removal of β2-microglobulin by mild acid treatment so that most surface MHC-I molecules become free heavy chains reveals F_1-ATPase epitopes on MHC-I^+ cell lines. Ecto-F_1-ATPase is detected by immunofluorescence on primary fibroblasts which express moderate levels of MHC-I antigens. Up-regulation of MHC-I on these cells following IFN-γ and/or TNF-α treatment induces a dose-dependent disappearance of F_1-ATPase epitopes. Finally, biotinylated F_1-ATPase cell surface components co-immunoprecipitate with MHC-I molecules confirming the association of both complexes on Raji cells. Confocal microscopy analysis of MHC-I and ecto-F_1-ATPase β chain expression on HepG2 cells shows a co-localization of both complexes in punctate membrane domains. This demonstrates that the TCR target F_1-ATPase is in close contact with MHC-I antigens which are known to control Vγ9/Vδ2 T cell activity through binding to natural killer inhibitory receptors.

Keywords: Innate immunity; Gamma-delta lymphocytes; ATP synthase; MHC-class I antigens

1. Introduction

T lymphocytes expressing an antigen receptor (TCR) of the γδ type represent a small fraction (1–5%) of T cells in human blood and lymph nodes although they may be more abundant in mucosae-associated lymphoid tissues. Striking features of these lymphocytes include an activated cell phenotype and a biased TCR V gene repertoire varying with their tissue distribution, so that the vast majority of human peripheral γδ cells express a Vγ9/Vδ2 TCR variable region gene combination (for reviews, see Hayday, 2000; Pennington et al., 2005). These Vγ9/Vδ2 T cells proliferate in vivo and in vitro in response to bacterial non-peptidic phosphorylated antigens termed phosphoantigens (PAg) in a TCR-dependent manner. To date the most powerful natural phosphoantigen is hydroxy-dimethylallyl pyrophosphate (HDMAPP), a metabolite of the 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway of isoprenoid biosynthesis in plants and bacteria (Bukowski et al., 1998; Morita et al., 2007; Poupot and Fournie, 2004). How the bacterial PAgs produced by infected cells are presented to T cells is poorly understood although evidence exist that this requires a cell–cell contact which can be between sister Vγ9/Vδ2 T cells

Abbreviations: ApoA1, apolipoprotein A-I; β2m, beta-2-microglobulin; α-F_1, β-F_1, alpha and beta subunits of F_1 domain of ATP synthase; EBV-B, Epstein–Barr virus-immortalized B cell line; TAP, transporter associated with antigen processing; MHC-I, MHC-class I antigens; iNKR, inhibitory natural killer cell receptor for MHC-class I antigens

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(Bonneville and Fournie, 2005; Green et al., 2004; Lang et al., 1995; Morita et al., 1995).

Vγ9/Vδ2 T cells are also activated by several tumoral cell lines including hematopoietic tumors Daudi (Burkitt's lymphoma), RPMI8226 (myeloma) and K562 (erythroleukemia), and solid tumors such as renal cell carcinomas which are frequently found to be associated with Vγ9/Vδ2 T cell infiltrates in vivo (Zocchi and Poggi, 2004). Recent findings implicate ubiquitous endogenous phosphoantigens (isopentenyl pyrophosphate and dimethylallyl-pyrophosphate) in Vγ9/Vδ2 T cell activation by tumors. Indeed, their accumulation or depletion following modulation of the mevalonate pathway by aminobisphosphonates or statins leads to an increase or decrease of the stimulatory activity, respectively (Gober et al., 2003). Again, activation by these metabolites is likely to require some form of antigen presentation by unknown structures.

We recently described the presence of components of the mitochondrial ATP synthase on the surface of tumor cells with stimulatory activity for Vγ9/Vδ2 lymphocytes (Scotet et al., 2005). This structure, referred to as ecto-F_1-ATPase (F_1) binds a soluble form of apolipoprotein A-I (ApoA1) so that an F_1-ApoA1 complex is frequently detected on the surface of stimulatory tumors. Moreover, purified soluble forms of F_1 and ApoA1 both specifically bind to a recombinant soluble Vγ9/Vδ2 TCR and the F_1-ApoA1 complex is stimulatory for Vγ9/Vδ2 T cells when immobilized on polystyrene beads. These components could be involved in endogenous phosphoantigen presentation although there is still no direct evidence for this.

Cell surface expression of F_1 is not confined to tumor cells as several studies have shown the presence and enzymatic activity of F_1 on hepatocytes, keratinocytes or endothelial cells (Burrell et al., 2005; Champagne et al., 2006; Martinez et al., 2003; Moser et al., 1999). In the present paper, we provide evidence that F_1 is more frequently expressed on the surface of tumoral and non-tumoral cells than previously thought on the basis of immunofluorescence analyses and that a close association exists between F_1 and Major Histocompatibility Complex class I antigens (MHC-I). Indeed, when these antigens are highly expressed, they can prevent F_1 detection. Knowing that Vγ9/Vδ2 T cell activation is strictly controlled by activatory and inhibitory receptors for MHC-I antigens (Fisch et al., 1997; Halary et al., 1997; Poccia et al., 1997; Trichet et al., 2006), this has important functional implications regarding the regulation of their anti-tumoral and inherent anti-self reactivity.

2. Material and methods

2.1. Tumor cell lines, cultures and antibodies

Cell lines are from the ATCC except the human β2m Daudi transfectant (Anne Quillet, Toulouse, France) and RCC7 (Anne Caignard, Villejuif, France). 721.221 (MHC-I deficient; Shimizu and De Mars, 1989) and Awells (IHW#9090) are EBV-B cell lines. ST-EMO (EBV-B) and ST-F1 (primary fibroblast) are derived from a TAP-deficient patient (provided by Henry de la Salle, Strasbourg, France). HD-F1 are normal primary foreskin fibroblasts obtained from Purpan Hospital pediatric surgery department (Toulouse, France). RMA is a murine T lymphoma and RMA-S is its TAP-deficient variant (Ljunggren and Karre, 1985). Cell lines were cultivated in RPMI 1640 medium except for HepG2, primary fibroblasts and RCC7 (DMEM), supplemented with FCS, glutamine and antibiotics.

The monoclonal anti-α (clone 7H10, IgG_{2b}) and anti-β (clone 3D5, IgG_1) F_1-ATPase antibodies used for immunofluorescence were from Molecular Probes. For immunoblotting studies, anti-α-F_1 (clone 51, IgG2a) and anti-β-F_1 (clone 10, IgG_1) were from BD Biosciences. The anti-cytochrome *c* antibody (clone 7H8.2C12, IgG_{2b}) was from R&D Systems. The anti-HLA-DR FITC antibody (Immu-257, IgG_1) and isotype controls were from Beckman-Coulter. The anti-CD19 antibody (clone LT19, IgG_1) was from Exbio (Prague, Czech Republic). The HC10 (anti-MHC-I free heavy chain) antibody (Stam et al., 1990) was kindly provided by Dr. H.L. Ploegh (Cambridge, MA). The W6/32 hybridoma (anti-HLA-class I) was obtained from ATCC. The anti-H-$2K^bD^b$ was from Cedarlane Laboratories.

2.2. Induction of MHC-I expression by cytokines and flow cytometry analysis

Fibroblast cells and other adherent cell lines were plated the day before treatment on six-well dishes. Control Daudi cells were grown in suspension. For MHC-class I induction experiments, IFN-γ and/or TNF-α (Biosource) were added to cell cultures (48 h). Adherent cells were harvested using ice-cold PBS containing 10 mM EDTA. Cells were then washed with PBS 5% FCS (FACS medium). Staining and washing were performed in FACS medium using a standard indirect immunofluorescence procedure. Primary mAbs and isotypic controls were used at the concentration of 5–10 μg/ml. Secondary staining was performed using polyclonal goat $F(ab)'_2$ anti-mouse IgG-FITC (Dako Cytomation). Data were acquired on a FACScan flow cytometer (BD Biosciences).

2.3. Disruption of cell surface MHC-I by acid treatment

The procedure described by Dong et al. (2003) was used with minor modifications. Briefly, cells were harvested, washed once in PBS and incubated for 1 min on ice in 1 ml of citrate–phosphate buffer (66 mM NaH_2PO_4, 131 mM citric acid, 1% BSA, pH 3) or PBS as a control. pH was neutralized by the addition of 9 ml of PBS (pH 13), and cells were pelleted, washed once with FACS medium, and stained immediately for flow cytometry analysis or put back in culture.

2.4. Streptavidin pull-down and co-immunoprecipitation experiments

Biotinylation and precipitation of cell surface proteins was performed according to Altin and Pagler (1995) with few modifications using the membrane-impermeant NHS-LC-biotin reagent (Pierce, 0.25 mg/ml). Co-precipitation of F_1 components with MHC did not require cross-linking. After cell lysis (150 mM NaCl, 1% Triton X-100, Tris 20 mM, pH 7.6, anti-proteases) and centrifugation (10,000 g, 15 min, 4 °C)