NEOPLASIA
www.neoplasia.com

Volume 13 Number 9 September 2011 pp. 822–830 822

Human Leukocyte Antigen E Contributes to Protect Tumor Cells from Lysis by Natural Killer Cells[1,2]

Elisa Lo Monaco*, Elisa Tremante*, Cristina Cerboni†, Elisa Melucci‡, Leonardo Sibilio*,3, Alessandra Zingoni†, Maria Rita Nicotra*, Pier Giorgio Natali§ and Patrizio Giacomini*

*Laboratory of Immunology, Regina Elena Cancer Institute CRS, Rome, Italy; †Department of Molecular Medicine, University "La Sapienza," Rome, Italy; ‡Department of Pathology, Regina Elena Cancer Institute, Rome, Italy; §Consorzio Interuniversitario Nazionale per la Bio-Oncologia Laboratories, University "Gabriele d'Annunzio," Chieti, Italy

Abstract

The nonclassic class I human leukocyte antigen E (HLA-E) molecule engages the inhibitory NKG2A receptor on several cytotoxic effectors, including natural killer (NK) cells. Its tissue distribution was claimed to be wider in normal than in neoplastic tissues, and surface HLA-E was undetectable in most tumor cell lines. Herein, these issues were reinvestigated taking advantage of HLA-E–specific antibodies, immunohistochemistry, and biochemical methods detecting intracellular and surface HLA-E regardless of conformation. Contrary to published evidence, HLA-E was detected in a few normal epithelia and in a large fraction (approximately 1/3) of solid tumors, including those derived from HLA-E–negative/low-normal counterparts. Remarkably, HLA-E was detected in 30 of 30 tumor cell lines representative of major lymphoid and nonlymphoid lineages, and in 11 of 11, it was surface-expressed, although in a conformation poorly reactive with commonly used antibodies. Coexpression of HLA-E and HLA class I ligand donors was not required for surface expression but was associated with NKG2A-mediated protection from lysis by the cytotoxic cell line NKL and polyclonal NK cells from healthy donors, as demonstrated by antibody-mediated relief of protection in 10% to 20% of the tested target-effector combinations. NKG2A-mediated protection of additional targets became evident on NK effector blocking with antibodies to activating receptors (DNAM-1, natural cytotoxicity receptors, and NKG2D). Thus, initial evidence that the long-elusive HLA-E molecule is enhanced by malignant transformation and is functional in tumor cells is presented here, although its importance and precise functional role remain to be addressed in the context of a general understanding of the NK ligand–receptor network.

Neoplasia (2011) 13, 822–830

Abbreviations: CTL, cytotoxic T lymphocyte; DNAM-1, DNAX accessory molecule 1; E/T, effector-target; HLA, human leukocyte antigen(s); IFN, interferon; ILT, immunoglobulin-like transcript; KIR, killer immunoglobulin-like receptor; NCR, natural cytotoxicity receptor; NHEM, normal human epidermal melanocyte; NK, natural killer; PBMC, peripheral blood mononuclear cell; TcR, T-cell receptor; β_2m, β_2-microglobulin

Address all correspondence to: Dr. Patrizio Giacomini, Laboratory of Immunology, Regina Elena Cancer Institute CRS, Via delle Messi d'Oro 156, 00158 Rome, Italy.
E-mail: giacomini@ifo.it

[1]Work supported by the Italian Ministries of Public Health (C.C., A.Z., and P.G.), University and Research (MIUR) (C.C. and A.Z.), AIRC (C.C., A.Z., P.G.N., and P.G.), and the Center of Excellence (BEMM) Rome, Italy (C.C. and A.Z.). The authors have no conflict of interest.
[2]This article refers to supplementary materials, which are designated by Tables W1 to W4 and Figures W1 to W6 and are available online at www.neoplasia.com.
[3]Present address: Istituto Biochimico Italiano (IBI) Lorenzini, Via di Fossignano 2, 04011 Aprilia, Italy.
Received 6 December 2010; Revised 22 July 2011; Accepted 28 July 2011

DOI 10.1593/neo.101684

Introduction

Human leukocyte antigen E (HLA-E) is a nonclassic class I molecule recognized by natural killer (NK) cells, CD8 cytotoxic T lymphocytes (CTLs), and a more recently described subset of CD8 effectors with memory phenotype, called by some authors NK-CTLs [1–4]. NK cells and certain CTL subsets engage cell surface HLA-E through heterodimeric lectin-like receptors, both inhibitory (CD94/NKG2A) and activating (i.e., CD94/NKG2C). NK-CTLs primarily engage HLA-E through (oligo)clonally rearranged T-cell receptor (TcR) and lyse target cells, but they may also express NKG2A [1–4].

Inhibition through NKG2A is possibly the most thoroughly understood function of HLA-E. It requires the stabilization of the HLA-E heavy chain through association with its light-chain subunit (β_2m) and short peptide ligands cleaved from the signal sequences of "permissive" class I alleles (the classic HLA-A, -B, and -C and the nonclassic HLA-G heavy chains), with the aid of class I–dedicated chaperones such as TAP and tapasin (reviewed in Rodgers and Cook [5]).

Coexpression of HLA-E and permissive alleles, crucial to this mechanism of ligand donation/stabilization, is thought to protect the conceptus from a maternal hemiallogeneic response [6] and prevent the inappropriate recognition of somatic self [7], but HLA-E may also favor immunoevasion. For instance, some viral genomes encode proteins acting as surrogate donors of HLA-E ligands [8,9], and ovarian carcinoma cells were shown to express increased levels of the ligand donor HLA-G as a result of interferon γ (IFN-γ) treatment [10]. However, because IFN-γ also upregulates antigen-presenting HLA-A, -B, and -C molecules, that is, a full set of major activating T-cell ligands, it is difficult to predict the final outcome (evasion or tumor control) in this and in similar [11] situations.

Unfortunately, the critical issue of whether HLA-E levels differ between normal and neoplastic tissues remains largely not addressed. For instance, immunohistochemistry detected HLA-E at several extraplacental locations, including normal white blood cells, liver, skin, and lung, but the reactive cell types were not specified [12]. Expression in the skin was subsequently confirmed [11], and HLA-E was also detected in certain endothelia but not in the few tested glandular epithelia [13]. As to biochemistry and flow cytometry studies, the commonly used 3D12 and MEM antibodies detected HLA-E polypeptides in the soluble extracts and/or on the surface of only 10 of 37 [14] and 4 of 31 [15] tumor cell lines. To complicate interpretations, HLA-E transcripts could be detected in the absence of HLA-E polypeptides [15], and HLA-E polypeptides were detected at an intracellular location but not on the cell surface [11].

On the basis of the available data, one might conclude that HLA-E is expressed in an undefined, possibly wide, range of normal tissues, but only in a few tumor cells in culture, either constitutively (seldom) or (possibly more often) following IFN-γ treatment, providing a weak rationale to investigate its function in tumor cells. Possibly for this reason, there are, to our knowledge, few published studies on this topic [11,16].

A more recent study of ours may help to reinterpret some of these results. In this study [17], it was shown that 3D12 and the MEM antibodies [11,13–15,18] selectively bind a subpopulation of unfolded HLA-E molecules free of β_2m, whereas biochemical approaches, among which the most effective is a reverse biotin labeling method, detect surface HLA-E regardless of conformation.

Using the HLA-E–specific [17] MEM-E02 antibody, we report herein the tissue distribution of HLA-E in normal nonlymphoid tissues and their malignant counterparts. Through biotin labeling and cytotoxicity assays, we measure surface HLA-E expression and assess for the first time the NK-inhibitory function of HLA-E constitutively expressed under the control of its own promoter in untransfected neoplastic cell lines, in the presence and absence of permissive alleles. The findings reported herein reconcile previous conflicting results, alleviate some theoretical inconsistencies, and bear several implications in tumor immunology.

Materials and Methods

Immunohistochemistry

Neoplastic tissues from patients (free from therapy) undergoing surgery were obtained upon written consent following the recommendations of the latest (March 1, 2006) Regina Elena Institute Ethical Committee Official Guideline. For further details, see Supplemental Materials and Methods.

Cell Lines

The 221B lymphoblastoid cell line and its transfectants, namely, 221.AEH [19], 221.G1, 221.B15 [20], and 221.B*0702 [21], were obtained through the courtesy of different investigators and/or the collaborative efforts of the HLA-G and -E Workshops (see acknowledgments). Epstein-Barr virus–immortalized B lymphocytes (EBV-B) and tumor cell lines are listed in the Supplemental Materials and Methods along with their HLA-A, -B, and -C typing. Some of these are early passage (<10 subcultures) tumor cells previously established [22]. Normal human epidermal melanocytes (NHEMs) were purchased from Lonza (Walkersville, MD). HLA-E genotyping was obtained by direct sequencing of genomic DNA, as described [17].

Biochemical Methods

The mouse monoclonal antibodies MEM-E/02, MEM-E/06, MEM-E/07, and MEM-E/08 [13,17,18] are all from Exbio, Prague, Czech Republic; 3D12, 4D12 [14,23], W6/32 [24], Namb-1 [25], L31 [26], and a polyclonal to ERp57 were used in previous publications of ours [22,26–29]. The reverse surface biotin labeling method is described [17].

Flow Cytometry

Tumor cells and peripheral blood mononuclear cells (PBMCs)/purified NK cells were stained on ice with either fluorochrome-labeled antibodies or with a predetermined optimal (10 μg/ml) concentration of primary antibody/chimeric immunoglobulin (Ig). In the latter case, primary antibody binding was revealed by fluorescence isothiocyanate–labeled rabbit antibodies to either mouse or human Ig (Dako, Glostrup, Denmark). Isotype-matched control antibodies, or a chimeric Ig of irrelevant specificity, were included as negative controls. Specifically bound fluorescence was immediately analyzed without fixation by a FACScan flow cytometer (Becton, Dickinson & Co, Mountain View, CA). Antibodies to MICA (159227), MICB (MAB), ULBP1 (170818), ULBP2 (165903), ULBP3 (166510), NKG2A (131411), NKG2C (134591), NKG2D (149810), DNAX accessory molecule 1 (DNAM-1/CD226) (102511), NKp30 (210845), NKp44 (253415), NKp46/CD335 (195314), and recombinant human Fc chimeras to activating immune receptors DNAM-1-Fc, NKp30-Fc, NKp44-Fc, and NKp46-Fc were from R&D Systems (Minneapolis, MN). SKII.4 to polyovirus receptor (CD155) was from Dr Marco Colonna. Antibody to Nectin-2 was from BD Pharmingen (San Jose, CA). Antibodies to CD3 (UCHT1) and CD56 (MOC-1) were from Dako. Antibodies