ELSEVIER

Identification of HLA-DQα and -DRβ Residues Associated With Susceptibility and Protection to Epithelial Ovarian Cancer

Dimitri S. Monos, John Pappas, Eleni E. Magira, John Gaughan, Richard Aplenc, Lazaros Sakkas, Ralph Freedman, John D. Reveille, and Chris D. Platsoucas

ABSTRACT: Substantial evidence has been accumulated suggesting that T cells in patients with epithelial ovarian carcinoma (EOC) exhibit an antigen-driven immune response directed against the tumor cells. In the context of human leukocyte antigen (HLA), this suggests its possible involvement in the disease. Therefore, we examined the distribution of the HLA-DRB1*, -DQA1*, and -DQB1* alleles in 47 patients with EOC and 67 healthy Caucasian women. The frequency of D^{70} and E^{71} polymorphic residues of the DRB1 alleles was significantly reduced in EOC patients versus controls ($pD^{70}E^{71} = 0.009$), suggesting a protective role against the disease. The DQα residues R^{52} and $Y^{11}R^{55}$ were increased in the patients ($p = 0.008$ and 0.012, respectively). Because residues 11 and 55 participate in the formation of pocket 1, they may be functionally important amino acid positions that influence disease susceptibility. The frequency of the DQα susceptibility epitope ($R^{52}Y^{11}R^{55}$) among the DRβ$D^{70}E^{71}$-positive EOC patients was increased when compared with DRβ$D^{70}E^{71}$-positive controls (EOC, 100%; control, 52%; $p = 0.028$). Among individuals without the DQα susceptibility epitope, the distribution of DRβ$D^{70}E^{71}$-positive cases was significantly different between EOC patients and controls (EOC, 0%; control, 60%; $p = 0.039$). Therefore, it appears that the presence of DQα susceptibility elements overrides the protective effect of the DRβ$D^{70}E^{71}$ epitope and suggests an interactive relationship between DRβ and DQα epitopes that may be of importance for disease susceptibility. Because positions DRβ 70,71 and DQα 52 have been implicated in immunologic diseases, it is likely that besides being critical for T-cell recognition, they may also play a role in T-cell development and acquisition of the T-cell repertoire. *Human Immunology 66, 554–562 (2005).* © American Society for Histocompatibility and Immunogenetics, 2005. Published by Elsevier Inc.

KEYWORDS: HLA-DQα; HLA-DRβ; ovarian cancer; disease susceptibility; protection

ABBREVIATIONS

EOC	epithelial ovarian carcinoma
HLA	human leukocyte antigen
IDDM	insulin-dependent diabetes mellitus
PCR	polymerase chain reaction
RA	rheumatoid arthritis
rIL	recombinant interleukin
SSP	sequence-specific primer
TIL	tumor-infiltrating lymphocyte

INTRODUCTION

Epithelial ovarian carcinoma(EOC) is the fourth leading cause of cancer-related deaths among women in the United States [1–3]. Although recent progress has been made, the 5-year survival rate for these patients is low among gynecologic malignancies [1–3]. Human tumor cells in a number of malignancies, including EOC, are recognized as nonself by the immune system and elicit an

From the Department of Pediatrics (D.S.M., E.E.M., R.A.), University of Pennsylvania School of Medicine, Philadelphia, PA, USA; the Department of Pathology and Laboratory Medicine, The Children's Hospital of Philadelphia, Philadelphia, PA, USA; Laboratory of Human Biology and Genetics (D.S.M.), Democritus University of Thrace, School of Medicine, Alexandroupolis, Greece; Departments of Microbiology and Immunology (J.P., L.S., C.D.P.) and Physiology (Biostatistics) (J.G.), Temple University School of Medicine, Philadelphia, PA, USA; Department of Gynecologic Oncology (R.F.), The University of Texas, M.D. Anderson Cancer Center, Houston, TX, USA; and Division of Rheumatology and Molecular Immunology (J.D.R.), University of Texas Medical School at Houston, TX, USA.

Address reprint requests to: Dr. Chris D. Platsoucas, Department of Microbiology and Immunology, Temple University School of Medicine, 3400 N Broad Street, Philadelphia, PA 19140; Tel: (215) 707-7929; Fax: (215) 829-1320; E-mail: chris.platsoucas@temple.edu.

J.P. and E.E.M. contributed equally to this work.

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immune response (reviewed in [4]). Ovarian tumors are infiltrated by tumor-infiltrating lymphocytes (TILs), which are mostly composed of T cells, and they may represent an immune response of the host to the tumor (reviewed in [4]). Tumor-infiltrating lymphocytes are present in both malignant ascites and solid tumors from patients with EOC [5, 6]. The presence of TILs within tumor-cell islets correlates with improved clinical outcome and increased expression of cytokines and chemokines within the tumor in patients with advanced ovarian carcinoma [7].

We have previously developed and expanded in low concentrations of recombinant interleukin-2 (rIL-2) T-cell lines and clones from TILs from patients with EOC that exhibit cytotoxicity [8–11] or cytokine production [12] primarily restricted to autologous tumor cells. These T-cell lines and clones were mostly $CD8^+$ and did not lyse allogeneic ovarian or other tumor cells, autologous normal cells, or K562 cells [8–11]. These findings have been confirmed by others [13–16]. Amplification by the nonpalindromic adaptor polymerase chain reaction (PCR) [17–20] followed by cloning and sequencing of β-chain T-cell receptor transcripts from solid tumor specimens from patients with EOC revealed substantial proportions of identical β-chain T-cell receptor transcripts [21], demonstrating the presence of monoclonal/oligoclonal populations of T cells in ovarian carcinoma TILs. It is very likely that these T cells have undergone antigen-driven proliferation and clonal expansion *in situ* in the tumor, in response to as yet unidentified antigens.

Tumor antigens that elicit antigen-specific major histocompatibility complex–restricted T-cell responses have been identified, initially in melanoma (reviewed in [4, 22, 23]). Peptides derived from these antigens are recognized by T cells in association with human leukocyte antigen (HLA) class I or class II. In EOC, the expression of tumor antigens such as MAGE, GAGE, and BAGE [24, 25], suppressor genes such as p53 [26], protooncogenes such as Hert-2/neu [27], and others [28] has been documented. However, lineage-specific tumor antigens, such as MART-1 and gp100 in melanoma, which are expressed in all tumor cells, have not yet been identified in EOC. Both HLA class I and II immune responses are important for the development of tumor vaccines for EOC [29–31]. Progress in our understanding of T-cell immune responses in terms of identification of specific epitopes of tumor antigens in EOC that are processed and presented in the context of specific HLA class I or II is undoubtedly important for the optimization of T-cell therapies and the development of effective tumor vaccines. We have demonstrated that HLA class I expression on human EOC cells correlates well with T-cell infiltration of the tumor *in vivo* and T-cell expansion *in vitro* in low concentrations of rIL-2 [32]. However, there are no studies in EOC on the HLA isotypes, alleles, or epitopes associated with the disease itself. Because of the importance of developing T-cell–based immunotherapy and the opportunities provided by the identification of tumor antigens, it is timely to evaluate the precise restriction elements of the HLA that determine these responses.

In the present study, an EOC and a control population are analyzed for the HLA-DRB1, HLA-DQA1, and HLA-DQB1 alleles. Comparisons of the allele frequencies in the two groups revealed the presence of DRβ and DQα residues associated with protection and susceptibility, respectively. An interactive relationship between DR and DQ isotypes that influences susceptibility or protection is proposed. These findings form the basis for dissecting the molecular interactions involved in the process of developing T-cell responses in EOC.

MATERIALS AND METHODS

Populations Used in the Study

Data from 47 Caucasian patients with a confirmed diagnosis of EOC were studied. Specimens were obtained from the Department of Gynecologic Oncology of the M.D. Anderson Cancer Center, under the approval of the M.D. Anderson Cancer Center Institutional Review Board for research involving human subjects. Sixty-four Caucasian women, all healthy blood donors with no history of cancer, served as controls. These studies were also approved by the Institutional Review Board of Temple University Hospital.

DNA Isolation

Genomic DNA was extracted from peripheral blood mononuclear leukocytes with phenol chloroform–isoamyl alcohol. The DNA was precipitated with ethanol as described elsewhere [33].

DNA-Based Typing

Locus- and allele-specific amplification of genomic DNA were performed for DRB1-, DQA1-, and DQB1-associated alleles. A 270–base pair amplified DNA product was verified by electrophoresis in a 2% agarose gel containing ethidium bromide and visualized under ultraviolet light. Hybridization was performed as described elsewhere by using a panel of sequence-specific oligonucleotide probes [34–36]. The sequence-specific primer (SSP)-PCR using ARMS™ (Zeneca Limited) technology was used for high-resolution HLA-DRB1, HLA-DQA1, and HLA-DQB1 typing. Depending on the low-resolution results of DRB1 and DQB1 typing, appropriate kits (Olerup SSP by Genovision and Biosynthesis) were chosen for subtyping of DRB1 and DQB1 locus (high-resolution typing). When sequence-specific