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γδ T cells recognize tumor cells via CDR3δ region

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Abstract

The principles governing γδ T cell specificity and diversity remain unclear due to lack of detailed structural analysis. To elucidate key structural basis of the specificity of γδ TCR for tumors, we analyzed the binding activities of synthesized TCR Vδ2 CDR3 peptides derived from tumor infiltrating lymphocyte (TIL) s in ovarian epithelial carcinoma (OEC) via biospecific interaction analysis approach, enzyme immunoassay and immunofluorescence assays. Besides, we used human CDR3δ grafted-Ig to repeat major tests. We found that synthesized OEC-derived CDR3δ peptides could bind specifically to tumor cell lines and tissues. CDR3δ-graft Ig showed a similar binding specificity with CDR3δ peptides, suggesting the determinant role of CDR3δ in antigen binding. Moreover, CDR3δ peptide-mediated binding specificity was blocked by pre-incubation with same peptide, which decreased the cytotoxicity of γδ T cells to OEC cells in vitro. Our finding indicates that CDR3δ peptide could mimic antigen-binding specificity of γδ TCR. Our strategy provides a novel, simple and convenient approach to investigate the binding activity and function of γδ TCR.

Keywords: γδ TCR; Tumor; CDR3

1. Introduction

The hallmark of the vertebrate immune system is its ability to mount responses virtually against any foreign entity, in the course of organism–environment interaction. During evolution, the vertebrates have developed four widely used mechanisms to recognize antigens. Pattern recognition of microbes by macrophages and dentritic cells through TLRs focuses on recognizing entities from different species, acting as sensors of pathogens (Medzhitov et al., 1997). Missing self-recognition of NK cells is responsible for detecting changes or loss of self-MHC sequence determinants caused by pathogenic assault or malignancy (Karre et al., 1986). The two recognition mechanisms above are employed by the innate immune system. In adaptive immunity, the antigenic specificities of αβ T cells and B cells are targeted for countless foreign antigens by means of high diversities of antigen-receptor repertoire (Davis and Chien, 2003). Although γδ TCR is structurally similar to αβ TCR and B cell receptor, consisting of heterodimer chains and containing a membrane-distal variable and membrane proximal constant Ig super family domain, their function is believed to serve as an early defense against certain commonly encountered microbes at epithelial barriers and recognize antigens directly in innate immunity (De Libero, 2000; Chien and Hampl, 2000; Hayday, 2000). However, the exact biological significance of γδ T cells is still puzzling; one of the important reasons is the less understanding of their antigenic recognition mechanism. Therefore, it is necessary to explore the antigen-binding specificity of γδ T cells. Theoretically, the diversity of the γδ TCR repertoire (10^{18} unique junctions) is greater than that of the αβ TCR (10^{15} unique junctions). However, the findings are adverse. First, the diversity conferred by pairing of germline-encoded V domains is limited (Davis and Bjorkman, 1988). Second, only a few ligands for γδ TCR have been identified so far. It raises a possibility that γδ T cells may have rather limited diversity of TCR repertoire.

It is very important to clarify the specificity of γδ T cells, which may depend upon CDRs of γδ TCR. The putative antigen-binding site of the γδ TCR is primarily formed from three

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CDRs contributed by each V_γ or V_δ domain. Both CDR1 and CDR2 regions are encoded by germline V genes, while the CDR3 region is formed by somatic rearrangement of V (D) and J fragments. Sequence diversity in antigen receptors is not evenly distributed among all six CDRs. The diversity is highly concentrated in one or two CDR3s. It had been proposed that the principal antigenic specificity of an immunoglobulin or a TCR is derived from its most diverse CDR3s (Davis et al., 1997). The investigations in αβ TCR recognition demonstrate that most of the energy of the interaction with MHC–peptide complex resides in the CDR3-peptide contacts, while the CDR1 and CDR2 regions make less energetically important contacts (Davis et al., 1998). For most antibodies, the V_H CDR3 makes the specific contacts with antigen, and the other CDRs provide only minor contributions to the energy of binding and specificity. Diversity in the CDR3 region of V_H is sufficient for many antibody specificities (Xu and Davis, 2000).

How CDR3s contribute to the diversity of γδ TCR? The γδ TCR has been called as "antibody-like" due to two main reasons: first, CDR3 length distribution of γ and δ chains correlates more with that of antibodies than with that of αβ TCR (Rock et al., 1994). Second, γδ TCR and antibody can recognize and bind to intact proteins without antigen presentation (Chien, 1997). Therefore, we hypothesized that the primary sequence of CDR3 in γδ TCR, especially CDR3δ, due to similarity to CDR3β and V_H CDR3 in gene composition, could serve as the key determinant for the specificity of antigen binding. γδT cells are capable of killing many autogeneic or allogeneic tumor cells. Therefore, tumor cell lines are ideal tools for the research of γδT cell-mediated specificity even though we do not know so much identified antigens on them.

We developed a biospecific interaction analysis approach, enzyme immunoassay and immunofluorescence assays to identify the features of interactions between the CDRs of γδ TCR and their targets. Briefly, we first synthesized CDR3δ peptides to mimic the antigen-binding specificity of γδ TCR. The CDR3δ gene sequences were derived from tumor infiltrating lymphocytes (TILs) with γδ TCR phenotype in ovarian epithelial carcinoma (OEC). We then applied surface plasmon resonance (SPR), CDR3-mediated enzyme immunoassay and CDR3-mediated immunofluorescence assay to determine two possibilities: (i) whether synthesized CDR3δ peptides could bind specifically to putative target cell lines/tissues; (ii) whether CDR3δ-graft human Ig containing the identical CDR3δ sequence have binding specificity similar to that of the synthesized CDR3δ peptide. Our data showed that CDR3δ peptide could bind specifically to tumor cells and tissues, but not normal ones. These results first suggest that our CDR3-based biospecific interaction analysis is successful. Such approach would facilitate structural study in γδ TCR binding specificity. Moreover, CDR3δ peptide-mediated binding specificity was blocked by pre-incubation with same peptide, which decreased the cytotoxicity of γδ T cells to OEC cells in vitro. Besides, CDR3δ-graft Ig showed a similar binding specificity with CDR3δ peptides, suggesting the determinant role of CDR3δ in antigen binding. From point of structural biological view, interactive pattern of CDR3δ and its putative epitopes might be linear and not rely on three-dimensional support. In terms of importance of CDR3δ region in antigen recognition of γδ TCR, such notion has been recently proved to be true in mice (Shin et al., 2005). Therefore, the critical role of CDR3δ in their antigen-binding specificity seems to be similar in humans.

2. Materials and methods

2.1. Cell lines and human tissue specimens

HO8910, 803, Hela, HepG2, K562 and RPMI8226 were obtained from the American Type Culture Collection. The human ovarian tumor cell line SKOV3 was a gift of Dr. Keng Shen (Department of Gynecology, The Peking Union Medical College Hospital, China). PBMCs were obtained from peripheral blood of healthy donors by density gradient centrifugation on Ficoll-Hypaque (Pharmacia). Fresh tumor and normal tissue specimens were obtained from the Peking Union Medical College Hospital. All of the tissue specimens from patients diagnosed by standard histopathological and immunohistochemical assay were collected prior to treatment with chemotherapy, radiotherapy or Chinese traditional medical therapy. All human studies were carried out according to proved guidelines by Peking Union Medical College.

2.2. Reverse transcription polymerase chain reaction and sequence analysis

Total RNA was isolated from ten OEC tissues with TRIzol reagent (Promega). cDNA was synthesized using oligo-dT (Promega) as primer and moloney murine leukemia virus reverse transcriptase (Promega) in the reverse transcription reaction and amplified by PCR using Vδ- and Cδ-specific primers as follows. The primers were TCRδV1: 5′-CAG CCT TAC AGC TAG AAG ATT CAG C-3′; TCRδV2: 5′-GCA CCA TCA GAG AGA GAT GAA GGG-3′; TCRδV3: 5′-TCA CTT GGT GAT CTC TCC AGT AAG G-3′; TCRδC 5′-AAA CGG ATG GTT TGG TAT GAG GC-3′. Amplified cDNA was cloned into the pGEM-T easy vector (Promega) and sequenced with the ABI automatic sequencer 377.

2.3. Peptide synthesis

Peptides were synthesized in the peptide synthesis facility of the Academy of Military Medical Sciences, China. MAB peptide derived from CDR3β of specific αβ T cell clones that recognize melanoma antigen recognized by T cells-1 (Mandruzzato et al., 2002) was synthesized as an irrelative peptide control (Table 1).

Table 1
Amino acid sequences of synthesized peptides

Peptide	V	D	J
OT1	CACD	SHGPSRLMMEGGLLG	TDKLIFGKG
OT2	CA	RKDLPINNWGIPRI	DKLIFGKG
OT10	C	TTGMILCLPPILLGLMMQ	KLIFGKG
OT3	CD	FPSHTFHSTGGHT	TDKLIFGKG
MAB	CASS	FEGLG	TEAFFGQG