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Induction of regular cytolytic T cell synapses by bispecific single-chain antibody constructs on MHC class I-negative tumor cells

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Abstract

Certain bispecific single-chain antibody constructs exhibit an extraordinary potency for polyclonal T cell engagement and target cell lysis. Here we studied the structural basis for this potency, using laser scanning confocal microscopy. Cytolytic human T cell synapses could be triggered either by addition of a specific peptide antigen or an Ep-CAM-/CD3-bispecific T cell engager (BiTE). Both kinds of synapses showed a comparable distribution of all protein markers investigated. Two other BiTEs constructed from different Ep-CAM-specific antibodies gave similar results. BiTEs could also induce lytic synapses between human T cells and a MHC class I-negative, Ep-CAM cDNA-transfected cell line resulting in potent target cell lysis. This shows that certain T cell recognition molecules on target cells are dispensable for synapse formation and BiTE activity, and suggests that BiTE-activated polyclonal T cells may ignore major immune evasion mechanisms of tumor cells in vivo, such as loss of MHC class I expression.
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1. Introduction

Among the various immune mechanisms the body can mount against tumor cells, cytotoxic T cell responses play a dominant role (Boon et al., 1994; Nagorsen et al., 2003). This is particularly evident from the growing number of T cell evasion mechanisms being discovered in late-stage tumor cells. Loss of MHC class I (Bubenik, 2003) and costimulatory molecules (Stopeck et al., 2000), overexpression by tumor cells of cytokines interfering with Th1 cell development (Gorelik and Flavell, 2001; Urosevic et al., 2001), of granzyme inhibitory serpins (Bladergroen et al., 2002), pro-apoptotic ligands (Dong et al., 2002; Lee and Ferguson, 2003), and a tryptophane-degrading enzyme (Uyttenhove et al., 2003) are examples of evasion mechanisms predominantly directed against cytotoxic T cells.

An elegant bypass for certain evasion mechanisms could be the direct recruitment of T cells to tumor cells via bispecific antibodies designed to bind a common T cell signaling molecule and, simultaneously, a surface molecule on tumor cells (Segal et al., 1999). Ideally, this will lead to a potent polyclonal T cell response against all tumor cells bearing the surface antigen, which should be uniquely expressed or accessible on tumor cells but not on normal cells. Countless efforts were made to design such bispecific antibodies but few met the requirements of target-dependent cytotoxicity, decent productivity, independence on costimulation, and necessary potency. One kind of bispecific antibodies are bispecific T cell engagers (BiTEs) (Baeuerle et al., 2003). BiTEs are small recombinant antibody constructs based on two tandemly arranged single-chain antibodies. They show extreme cytotoxic potency in a low picomolar range, work at very low effector:target (E:T) ratios, only activate T cells in the presence of target cells, can be produced in sufficient amounts and show high anti-tumor activity in animal models (Dreier et al.,

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2003, 2002). The anti-CD3 domain of BiTEs recognizes the epsilon subunit of the CD3 complex. A BiTE molecule directed against the pan-B cell antigen CD19 and the CD3 complex (Loffler et al., 2003, 2000) is in early clinical development for the treatment of B cell malignancies. The basis for the extreme potency of BiTEs is not fully understood. Complete protection of target cells from BiTE-induced T cell cytotoxicity by a calcium chelator suggested a role of perforin (Loffler et al., 2000).

Immunological synapses were first described for the interface between naive CD4 T cells and antigen-presenting cells (APCs) (for a review see (Dustin, 2003)), where they are required for T cell activation. Immunological synapses are composed of a 'central supramolecular activation cluster' (cSMAC) (Anton van der Merwe et al., 2000) containing essential signaling molecules. cSMACs are surrounded by a domain of heterotypic cell adhesion molecule interactions referred to as 'peripheral supramolecular activation cluster' (pSMAC). Cytolytic synapses are formed between cytotoxic T lymphocytes (CTLs) and target cells. They resemble immunological synapses made between APCs and CD4 T cells, but contain in their cSMAC in addition to the signaling domain a secretory domain for the focused release of cytotoxic molecules like perforin and granzymes (Stinchcombe et al., 2001).

Here we performed a confocal immunofluorescence microscopy analysis of cytolytic synapses formed between human cytotoxic CD8 T cells and human tumor cell lines to gain insight into the structural basis for BiTE-induced T cell cytotoxicity. By choosing a particular T cell/target cell system we could induce the formation of cytolytic synapses either by a T cell peptide in the correct T cell receptor/MHC class I context, or, by addition of a BiTE recognizing both CD3 and the pan-carcinoma antigen epithelial cell adhesion molecule Ep-CAM (Armstrong and Eck, 2003; Balzar et al., 1999). We also studied whether BiTEs can induce functional cytolytic synapses between human T cells and target cells lacking MHC class I.

2. Materials and methods

2.1. Cell preparation and culture

The erbB2 peptide-specific CD8-positive T cell clone SuHie7 (Knabel et al., 2002) was cultivated in T cell medium (RPMI 1640, 7.5% human serum, 7.5% fetal calf serum, 1% non-essential amino acids, 1% penicillin/streptomycin, 1% glutamine, 1% sodium pyruvate, 200 U/ml IL2) and expanded by restimulation. Restimulations were performed with 1×10^5 SuHie7 T cells, 2.5×10^7 irradiated PBMC (5000 rad) and 5×10^6 irradiated EBV immortalized B cells (10,000 rad) as feeder cells in 25 ml T cell medium. IL-2 was substituted every two days at a concentration of 200 U/ml. After 14 days, T cells were harvested, washed and resuspended in T cell medium.

Human gastric carcinoma cell line KATO III (HLA A 0201, Ep-CAM+), human T cell/B cell hybridoma line T2 (HLA A 0201, Ep-CAM−) and K562 transfected with Ep-CAM cDNA (MHC I-, Ep-CAM+) (kindly provided by Prof. J. Johnson, Institute for Immunology, Munich) as target cells were cultured in RPMI 1640 with 10% fetal calf serum. Sequencing of the HLA A locus of KATO III was performed by the Laboratory for Immunogenetics (University of Munich, Germany). The HLA A surface expression on KATO III cells was confirmed by FACS analysis with a mouse anti-HLA A antibody (Dianova, Hamburg, Germany).

2.2. Antibodies and peptides

Generation, production and characterization of Ep-CAM-specific BiTE 1 was described previously (Mack et al., 1997, 1995). Ep-CAM BiTEs 2 and 3 were constructed and produced as described for Ep-CAM BiTE 1. They used other murine monoclonal antibodies specific for human Ep-CAM but share the CD3-binding single-chain antibody moiety with Ep-CAM BiTE 1.

Primary antibodies and their sources are listed in Table 1. We selected primary monoclonal mouse antibodies of different mouse IgG isotype such that chromophore-conjugated secondary antibodies with specificity for mouse isotypes could be employed for triple-color staining. As secondary antibodies, Alexa488, Alexa555, or Alexa647-conjugated, isotype-specific antibodies, goat anti-rabbit IgG, and donkey anti-rat IgG, were used as provided by Molecular Probes (Eugene, USA). Cy3-labeled rabbit anti-chicken IgG and Cy3-labeled donkey anti-rabbit IgG were from Jackson ImmunoResearch Laboratories (West Grove, USA). MOPC21 (Sigma, Munich, Germany) was used as an isotype control.

The erbB2 peptide with the sequence KIFGSLAFL corresponded to aa 369–377 of the human erbB2 tyrosine kinase. The peptide was obtained from Thermo Hybaid (Ulm, Germany) with a purity of over 95%.

2.3. Quantification of cell surface molecules

The number of CD3 molecules on the surface of SuHie7 T cells and of MHC class I antigen and Ep-CAM on Kato III cells was determined by the QIFIKIT® kit (DakoCytomation, Glostrup, Denmark) according to the manufacturer's instruction. Binding reactions of cells with murine monoclonal antibodies were performed in a volume of 50 μl volume with either 1,000,000 KATO III or 100,000 SuHie7 cells. CD3 was determined by the monoclonal antibody UCHT-1 (BD Biosciences, Heidelberg, Germany), MHC class I expression with monoclonal antibody HLA-A,B,C (BD Biosciences, Erembodegem, Belgium), and Ep-CAM with monoclonal antibody M79 (Micromet AG, Munich). Binding reactions with a large range of antibody concentrations were performed for 60 min at 4 °C followed by washing of cells twice in FACS buffer. For detection, a FITC-labeled goat anti-mouse antibody (DakoCytomatics, Glostrup, Denmark)

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