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Single cell force spectroscopy of T cells recognizing a myelin-derived peptide on antigen presenting cells

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ABSTRACT

T-cell recognition of peptide–MHC complexes on APCs requires cell–cell interactions. The molecular events leading to T-cell activation have been extensively investigated, but the underlying physical binding forces between T-cells and APCs are largely unknown. We used single cell force spectroscopy for quantitation of interaction forces between T-cells and APCs presenting a tolerogenic peptide derived from myelin basic protein. When T-cells were brought into contact with peptide-loaded APCs, interaction forces increased with time from about 0.5 nN after 10 s interaction to about 15 nN after 30 min. In the absence of antigen, or when ICAM-1-negative APC was used, no increase in binding forces was observed. The temporal development of interaction forces correlated with the kinetics of immune synapse formation, as determined by LFA-1 and TCR enrichment at the interface of T-cell/APC conjugates using high throughput multispectral imaging flow cytometry. Together, these results suggest that ICAM-1/LFA-1 redistribution to the contact area is mainly responsible for development of strong interaction forces. High forces will keep T-cells and APCs in tight contact, thereby providing a platform for optimal interaction between TCRs and peptide–MHC complexes.

1. Introduction

Initiation of immune responses often requires cellular interactions, but the underlying molecular mechanism and physical binding forces of the respective cell adhesion processes are only poorly understood. A notable example is the interaction between T lymphocytes and antigen presenting cells (APC). During their search for cognate antigen T cells continuously travel through the body, in particular through the secondary lymphoid organs such as lymph nodes and spleen. There, they move through the dense network formed by the dendritic cell (DC) dendrites, and scan the DCs for the presence of antigen, as revealed by 2-photon studies [1,2]. In the absence of antigen numerous brief and presumably low-avidity interactions with DCs take place. However, these weak interactions are not without consequences, as indicated by recent studies showing that in the absence of antigen T cells are continuously stimulated by TCR-mediated recognition of self MHC molecules on DCs, thereby inducing a basic T cell signalling level that is required for rapid responsiveness to foreign antigen [3]. In contrast, upon recognition of cognate antigen the T cells are arrested and form tight contacts with DC, lasting for more than 1 h, resulting in the formation of a so-called immune synapse (IS) and activation of the T cell [4,5]. Long-lasting contacts also occur between T cells and B cells which then can move as cell–cell conjugates through the lymph node [6], indicating that considerable binding forces must keep the cell couples together.

IS formation is triggered by interactions between TCR and MHC/peptide complexes resulting in an orchestrated assembly of signalling, adhesive, and scaffolding molecules at the cell contact zone [7]. A mature IS is characterized by a central part of the supramolecular activation cluster (c-SMAC), enriched in MHC and signalling molecules such as TCR and CD3. The c-SMAC is surrounded by a peripheral part (p-SMAC) enriched in adhesion molecules like ICAM-1 on the APC side, and its ligand LFA-1 on the T cell side. This clustering of membrane proteins is accompanied by re-organization of cytoskeletal proteins such as actin [8]. The $\alpha_L\beta_2$

Abbreviations: AFM, atomic force microscopy; SCFS, single cell force spectroscopy; MIFC, multispectral imaging flow cytometry; APC, antigen presenting cell; LFA-1, lymphocyte function antigen-1; ICAM-1, intercellular adhesion molecule-1; pMHC, peptide–MHC complex.

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integrin LFA-1 is present on resting T cells in a so-called closed and low-affinity form. Interaction of the TCR with MHC/peptide complexes will induce LFA-1 by inside-out signalling to undergo a conformational change and to adopt an open and high-affinity conformation. The high affinity LFA-1 molecules are then able to bind strongly to ICAM-1 molecules on APCs, thereby inducing the above-mentioned clustering of ICAM-1/LFA-1 complexes [9,10]. Sustained LFA-1 clustering and prolonged T-cell/APC interactions are then driven by calmodulin and the actin-binding protein L-plastin [11]. This mechanism of LFA-1 activation ensures that T cells bind firmly only to those APCs which present a cognate antigen.

IS formation is assumed to result in strong interaction forces between cells. Interaction forces can be precisely determined by atomic force microscopy (AFM) [12]. Being an extremely sensitive force sensor, AFM has been used to characterize the interaction force between individual molecules [13–15]. When applied as single cell force spectroscopy (SCFS) it also allows measuring the overall adhesion force between cells and substrate [13,16–18] or between pairs of cells [19,20], with the advantage that interaction of receptors and ligands can be studied in their physiological environment at the cell surface. In a previous study we have adapted AFM to the analysis of APC–T cell interactions and observed that IS development between APCs and T cells recognizing a hen–egg lysozyme (HEL) peptide was paralleled by an increase in binding forces between APCs and T cells [21]. Since these studies were the first ones to determine the interaction forces between T cells and APC, it was important to investigate another pair of T cells and APC. Whereas in the former study the T cells were specific for an immunogenetic HEL peptide, we selected here T cells recognizing a tolerogenic myelin-derived peptide, MBP Ac1-11.4Y. The natural MBP peptide Ac1-11 is known to induce experimental autoimmune encephalitis (EAE) in mice [22], the murine model for the autoimmune disease multiple sclerosis in humans. The altered peptide ligand Ac1-11.4Y contains at position 4 a lysine to tyrosine substitution which increases the affinity of the peptide for the MHC class-II molecule Au, and converts it into a tolerogenic peptide that can induce suppression of EAE [23,24]. As APC, Au-transfected fibroblasts were used that expressed or not ICAM-1, thereby allowing to determine the contribution of ICAM-1 to binding forces. Despite the fact that here and in the former study [21] very different T cell/APC pairs were employed, we obtained remarkably similar results with regard to the development of interaction forces.

2. Materials and methods

2.1. Cells and reagents

L.Au cells are Ltk$^-$ cells transfected with Au.MHC class II α and β chains, generously provided by the late Janeway. L.Au cells were supertransfected with a pABES-puro plasmid encoding murine ICAM-1 obtained from Vestweber and Cagua to generate L.Au.ICAM-1 cells. The T cell hybridoma 1934.4 expressing the Tg4 TCR with specificity for the myelin basic protein-derived peptide Ac1-11 was kindly provided by Wraith [22]. Cells were maintained in complete RPMI 1640 tissue culture medium, supplemented with 10% FCS. The anti-LFA-1 antibody M17.4 was purchased from BioXcell (USA). The MBP peptide Ac1-11 (ASQKRPSQRHG) and the high affinity altered peptide ligand Ac1-11.4Y (ASQYRPSQRHG) were produced by the peptide synthesis unit of the German Cancer Research Center.

2.2. Antigen presentation and cell conjugate assays

Antigen presentation assays were performed as described [25]. Briefly, 5×10^4 APCs were pulsed with peptide for 2 h, and incubated with 5×10^4 1934.4 T cells in triplicate in 96-well plates. After 40 h, IL-2 released by the T cells was measured by an europium-based fluorescence immunoassay. Cell conjugate assays were performed as reported [26]. Briefly, 1934.4 T cells were stained for 10 min by 37 °C with 0.5 μmol of CFSE (molecular probes). APCs were pulsed with 50 μg/ml of AC1-11 peptide, followed by labelling with 5 μmol of SNARF (InVitrogen). 10^5 cells of each population were mixed in 100 μl tissue culture medium, centrifuged for 1 min, and fixed after indicated times with 2% paraformaldehyde. Analysis was performed with a FACS Calibur instrument.

2.3. Single cell force spectroscopy

The basic principles of atomic force microscopy (AFM) and single cell force spectroscopy (SCFS) have been described elsewhere [12,19,21]. The results presented here were obtained by using a NanoWizard II AFM equipped with a Cellhesion™ module (JPK-Instruments, Berlin, Germany) which was operated on an inverted optical microscope (Axiovert 200, Zeiss, Göttingen, Germany). One day before the experiment, the L-cell APCs were seeded onto round glass coverslips with a diameter of 22 mm and incubated over night to allow firm adhesion. Ac1-11.4Y peptide loading was performed at least 2 h before use with a concentration of 50 μg/ml. Coverslips were then washed and mounted into the temperature controlled perfusion chamber (Biocell, JPK-Instruments) of the AFM and overlayed with Hank's buffered saline supplemented with 25 mM HEPES, pH 7.4. All AFM measurements were performed at 37 °C. Gold-coated arrow-shaped TL-1 cantilevers with a nominal spring constant of 0.03 N/m (Nanoworld, Neuchâtel, Switzerland) were functionalized prior to use as follows: cantilevers were plasma-cleaned and coated with 0.5 mg/ml biotinylated BSA in 0.1 M $NaHCO_3$ pH 8.8 over night at 37 °C. After washing three times with PBS they were incubated in 0.5 mg/ml streptavidin for 1 h at 37 °C. Cantilevers were washed again and incubated with 10 μg/ml of biotinylated anti-CD43 antibody for 1 h at 37 °C. Each coated cantilever was calibrated individually with the thermal-noise method using the AFM's built-in software routines. 1934.4 T-cells were washed twice with Hank's buffered saline supplemented with 25 mM HEPES and flushed into the Biocell. After sedimentation, a T cell was selected and attached to the anti-CD43 functionalized cantilever by touching it with a force of 1 nN for 10 s. Subsequently the cantilever with the attached T cell was retracted and then lowered onto an APC until a contact force of 1 nN was reached. Contact was maintained for different lengths of time. The retraction speed was set to 1 μm/s. For interaction times longer than 10 s, new T cells were attached to a cantilever for each measurement. Measurements were carried out in closed loop and constant height mode. Analysis of the resulting force–distance plots was done using the JPK-IP V. 3.3.4 software package (JPK-Instruments). Several experiments were performed with a NanoWizard I AFM as described previously [12] with comparable results.

2.4. Multi-spectral imaging flow cytometry

The APCs (L.Au or L.Au.ICAM-1) were loaded with 0 or 50 μg/ml MBP-peptide (Ac1-11.4Y) for 2 h at 37 °C. Thereafter, unbound peptide was removed and cells were resuspended in 250 μl culture medium at a density of 4×10^6/ml. 1934.4 T cells (4×10^6/ml) were added in an equal volume, mixed and centrifuged briefly ($200 \times g$ for 1 min). After removal of the supernatant the cells were resuspended in 150 μl culture medium and incubated in a controlled atmosphere (37 °C/5% CO_2) for the indicated time points. Thereafter, cells were fixed with 1.5% PFA and stained for LFA-1 (CD18-FITC) and TCR (TCRβ-biotin plus Streptavidin-PE-TXred), respectively. To highlight the nucleus, the cells were incubated with Hoechst 33342 in the presence of 0.05% saponin. After exten-