

CONTENTS

1. INTRODUCTION.....1

| | | |
|-----|-------------------------|----|
| 1.1 | T cell activation..... | 1 |
| 1.2 | Actin cytoskeleton..... | 7 |
| 1.3 | Cofilin..... | 11 |
| 1.4 | Aim of the study..... | 16 |

2. MATERIALS &

METHODS.....17

2.1. MATERIAL.....17

| | | |
|-------|--------------------------------------|----|
| 2.1.1 | General chemicals and materials..... | 17 |
| 2.1.2 | Cell culture materials..... | 19 |
| 2.1.3 | Inhibitors..... | 19 |
| 2.1.4 | Radiochemicals..... | 19 |
| 2.1.5 | Peptides..... | 20 |
| 2.1.6 | Technical equipment..... | 20 |
| 2.1.7 | Antibodies..... | 21 |
| 2.1.8 | Cells..... | 23 |

2.2

| | |
|--|----------|
| METHODS..... | 2 |
| 2.2.1 Stimulation of cells..... | 24 |
| 4 | |
| 2.2.2 Preparation of cytoplasmic and actin cytoskeletal fractions..... | 26 |
| 2.2.3 SDS-polyacrylamide gel-electrophoresis (SDS-PAGE)..... | 26 |
| 2.2.4 Non-equilibrium pH gradient electrophoresis (NEPHGE)..... | 28 |
| 2.2.5 Western-blotting..... | 29 |
| 2.2.6 Coupling of peptides to Penetratin and purification of the conjugates..... | 30 |
| 2.2.7 Enzyme-linked immunosorbent assay (ELISA)..... | 31 |
| 2.2.8 CD2 receptor cap formation and immunofluorescence microscopy..... | 33 |
| 2.2.9 Surface and intracellular staining for flow cytometry..... | 34 |
| 2.2.10 Proliferation assay..... | 35 |
| 2.2.11 Determination of Apoptosis..... | 36 |

3. RESULTS.....39

| | |
|---|----|
| 3.1 Activation of human peripheral blood T lymphocytes induces reversible association of cofilin with the actin cytoskeleton..... | 39 |
| 3.2 In activated T lymphocytes the dephosphorylated form of cofilin associates with the actin cytoskeleton..... | 41 |
| 3.3 Cofilin co-immunoprecipitates with cytoskeletal actin in activated T lymphocytes..... | 42 |
| 3.4 The PI3-kinase inhibitor wortmannin prevents dephosphorylation of cofilin and its association with the actin cytoskeleton in peripheral blood T lymphocytes but not in Jurkat T lymphoma cells..... | 44 |
| 3.5 Stimulation with PMA does not influence the association of cofilin with the actin cytoskeleton..... | 46 |

| | | |
|-----------|---|-----------|
| 3.6 | Wortmannin does not inhibit the constitutive association of cofilin with the actin cytoskeleton in Jurkat T lymphoma cells..... | 48 |
| 3.7 | The activation induced association of cofilin with the actin cytoskeleton is blocked by cofilin derived peptides..... | 49 |
| 3.8 | Penetratin coupled peptides efficiently internalize into living cells..... | 51 |
| 3.9 | The activation induced association of cofilin with the actin cytoskeleton <i>in vivo</i> is blocked by cell permeable cofilin derived peptides..... | 52 |
| 3.10 | Cofilin is involved in the process of the receptor cap formation..... | 54 |
| 3.11 | The interaction of cofilin with the actin cytoskeleton is required for T cell activation..... | 58 |
| 3.12 | The cofilin derived peptides inhibit CD2-mediated activation induced cell death (ACID)..... | 64 |
| 3.13 | Cofilin derived peptides do not influence CD95-induced apoptosis..... | 66 |
| 3.14. | Apoptosis induced via CD2 is inhibited by soluble anti-CD95 mAb..... | 68 |
| 3.15 | Cofilin derived peptides inhibit the expression of CD95 ligand..... | 70 |
| 4. | DISCUSSION..... | 72 |
| 4.1 | Reversible association of cofilin with the actin cytoskeleton following activation of human peripheral blood T lymphocytes..... | 72 |
| 4.2 | Involvement of PI3-kinase activity in the cofilin signaling pathway | 74 |
| 4.3 | Blockade of the activation induced interaction of cofilin with the actin cytoskeleton by cofilin derived peptides <i>in vivo</i> | 75 |
| 4.4 | Involvement of cofilin in the process of receptor cap formation..... | 76 |
| 4.5 | The interaction of cofilin with the actin cytoskeleton is an important step for T cell activation..... | 78 |

| | | |
|-----------|--|------------|
| 4.6 | Involvement of cofilin in CD2-mediated activation induced programmed cell death..... | 80 |
| 5. | SUMMARY..... | 8 |
| 6. | REFERENCES..... | 8 |
| 7. | ABBREVIATIONS..... | 104 |