The Human WD Repeat Protein WAIT-1 Specifically Interacts with the Cytoplasmic Tails of β7-Integrins*

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Integrins of the β7 subfamily, α4β7 and αEβ7, contribute to lymphocyte homing and to the development of protective or autoreactive immune responses at mucosal sites. The β7 subunits of integrins are considered important for regulation of stimulated cell adhesion and adhesion-dependent signal transduction. Using a yeast interaction trap screen, a human WD repeat protein, termed WAIT-1, was isolated that interacts with the integrin β7 cytoplasmic domain and is homologous to mouse EED and Drosophila ESC proteins. WAIT-1 also binds to the cytoplasmic domains of α4 and αE but not to those of α5 and αL subunits. Association of WAIT-1 and β7-integrin was confirmed by coprecipitation from transiently transfected 293 cells. The binding site for WAIT-1 was mapped to a short membrane-proximal region of the β7 cytoplasmic tail with Tyr-735 being of critical importance. Northern blot analysis revealed multiple WAIT-1-related transcripts with differential expression in circulating leukocytes, tissue-resident cells of diverse origin, and lymphoid malignancies. These results suggest that WAIT-1, together with the recently identified RACK1, may define a novel subfamily of WD repeat proteins that interact with distinct subsets of integrin cytoplasmic tails and may act as specific regulators of integrin function.

Expression of β7-integrins, α4β7 and αEβ7, is largely confined to leukocytes. Although α4β7-integrin is homogeneously expressed on natural killer cells, eosinophils, and naive T and B cells, its distribution on effector/memory T and B cells is restricted to a subset of gut-homing lymphocytes (1–6). Peripheral blood monocytes do not express β7-integrins, but α4β7 and αEβ7 are up-regulated upon stimulation of macrophage differentiation with phorbol ester or interferon-γ (7). Recent studies have indicated that, in addition, α4β7-integrin is induced on endothelial cells after treatment with proinflammatory mediators such as tumor necrosis factor (8). In contrast to α4β7, expression of the αEβ7-integrin is confined to a subset of gut-associated T lymphocytes and dendritic cells (4, 6, 9).

β7-Integrins are considered important for the development and function of gut-associated lymphoid tissues. Interaction of α4β7 with MadCAM-1 allows for tissue-specific migration of circulating lymphocytes into the lamina propria and Peyer’s patches of the gut (10, 11), whereas αEβ7 may retain intraepithelial lymphocytes within the gut epithelium through binding of E-cadherin on epithelial cells (12, 13). Lack of β7-integrins severely impairs the development of the gut immune system, as Peyer’s patches are absent or hypoplastic, and fewer intraepithelial lymphocytes are detected in β7-integrin-deficient mice (14). Moreover, gut-homing α4β7 CD4 T cells specifically harbor cellular memory for intestinal antigens, suggesting that αEβ7 helps to target and segregate intestinal versus systemic immune responses (15).

Integrins of the β7 family are involved in the development and/or progression of diseases such as colitis (16, 17), diabetic insulitis (18), and lymphoid malignancies. Integrin α4β7 has been implicated in the mucosal localization of malignant lymphomatous polyposis, a gastrointestinal variant of the mantle cell lymphoma (19), the homing of lymphocytes to the thymus of AKR mice resulting in thymic lymphoma (20), and is specifically expressed on mucosa-associated T and B cell non-Hodgkin’s lymphomas (21). Analysis of skin biopsies from patients with mycosis fungoides revealed that expression of integrin αEβ7 correlates with epidermotropism of infiltrating T cells and the stage of the disease (22, 23).

Together, these findings indicate that β7-integrins play an important role for physiological functions and pathological alterations of the immune system. The cytoplasmic tails of the β7 subunit are critical for integrin function because they regulate receptor avidity (24) and signaling (25). In this study, we applied a yeast interaction trap screening to identify cytoplasmic interactors for the human integrin β7 subunit. We describe the molecular cloning and characterization of the human WD repeat protein WAIT-1, which specifically interacts with the cytoplasmic tails of β7-integrin α and β subunits and is thus likely to be involved in regulation of either receptor avidity or signaling.

EXPERIMENTAL PROCEDURES

Antibodies and Cell Lines—The rat-anti-murine integrin β7 antibody M301 was kindly provided by Dr. P. Kilshaw (AFRC Institute of Animal Physiology and Genetics Research, Babraham, Great Britain). Polyclonal rabbit anti-IgA serum was purchased from Invitrogen. To generate antibodies specific for the mouse and human integrin α4 subunit, rabbits were immunized with the cytoplasmic peptide sequence NH2-RDDSVYINSSKDNDD-COOH (Eurogentech, Seraing, Belgium). Horseradish peroxidase-conjugated antisera directed against rat, rabbit, or human Ig were purchased from Dianova (Hamburg, Germany) or Bio-Rad (Munich, Germany).

The human lymphoma cell lines Jurkat and Ramos as well as 293 cells were obtained from ATCC (Rockville, MD). The C3H/He B cell lymphoma 3C13 expresses integrin α4 subunits but lacks detectable cell surface protein and RNA transcripts for integrin β1 and β7 subunits. 3C13 lymphoma cells were transduced by retroviruses containing the β7 subunit or control vector generating cell lines 38-β7 and 38-LXSN (26).

Construction of Yeast Expression Plasmids—The cytoplasmic tails...
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used in the interaction trap screen were generated by PCR. The primers used were 5'-GAGAGAAGGATCCACGGGCTTCGTCAGGTG-3' and 5'-GAGAGAAGGATCCACGGGCTTCGTCAGGTG-3' for the N-terminal port and 5'-GAGAGAAGGATCCACGGGCTTCGTCAGGTG-3' for the C-terminal port of β7 as well as forward and reverse primers used for amplification of the full-length β7 cytoplasmic tail. Partial sequences of the β7 cytoplasmic tail were generated as complementary primer pairs with flanking EcoRI (5') and SpfI (3') sites for subcloning into plex202. Primers contained a diagnostic NotI restriction site and a TAA stop codon. Primers used were 5'-AAATCCCGGCCTTCGTTGCACTATGAGGGGG-3' and 5'-TCGACGCAGAGGCACCGGGCGTG-3' for the membrane-proximal portion, 5'-AAATCCCGGCCTTCGTTGCACTATGAGGGGG-3' and 5'-TCGACGCAGAGGCACCGGGCGTG-3' for the membrane-distal portion, 5'-AAATCCCGGCCTTCGTTGCACTATGAGGGGG-3' and 5'-TCGACGCAGAGGCACCGGGCGTG-3' for the Y735A mutant, and 5'-AAATCCCGGCCTTCGTTGCACTATGAGGGGG-3' and 5'-TCGACGCAGAGGCACCGGGCGTG-3' for the Y735F mutant. All cytoplasmic tail constructs were verified by double-stranded nucleotide sequencing and subcloned into plex202 to generate fusion proteins with the lexA DNA binding portion.

**Yeast Interactor Screening**—The screening procedure for isolation of proteins interacting with the human integrin β7 cytoplasmic domain was essentially performed as described (28). The lexA expression vector was transformed into yeast strain EGY48 bearing the JK103 line and lysed in buffer containing 1% Triton X-100, 0.5% SDS, 100 mM NaCl, 0.5% deoxycholic acid, 0.1% SDS, 1 mM lactopyranoside. Bacteria were lysed by sonication in buffer containing 150 mM NaCl, 2 mM MnCl₂, 5 mM Tris, pH 7.5, and protease inhibitors at 4 °C for 30 min. Lysates were incubated overnight with 25 μl of sedimented protein A-Sepharose CL4B (Pharmacia), and adsorbed proteins were analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting.

**Northern Blot Analysis**—Total RNA from Jurkat and Ramos cells was separated on formaldehyde-containing agarose gels and blotted onto a nitrocellulose membrane. The Northern blot containing mRNA of various human tissues was purchased from CLONTECH. cDNA probes spanning the coding region of wait-1 or β7 (CLONTECH) were labeled using a random priming method (Amersham) using the high prime kit (Boehringer, Mannheim, Germany) and used for hybridization according to the manufacturer's instructions (CLONTECH).

**RESULTS**

**Molecular Cloning of WAIT-1, a Human WD Repeat Protein Interacting with β7-Integrin Cytoplasmic Domains**—The complete 52-amino acid cytoplasmic tail of the human integrin β7 subunit was fused in frame with the lexA DNA binding domain and used as a bait protein for yeast interaction trap screening of a Jurkat cDNA library. The integrin β7 bait protein was tested in yeast to exclude intrinsic activation of reporter genes, and expression was demonstrated by Western blot analysis of yeast lysates using polyclonal rabbit antibodies against lexA or the human integrin β7 cytoplasmic tail (data not shown). The interaction trap screen revealed 19 reproducibly positive cDNA clones as determined by their ability to allow growth of the auxotrophic yeast strain EGY48 on leucine-deficient medium and indigo blue staining of yeast in the presence of X-gal. Restriction enzyme analysis and nucleotide sequencing indicated that 14 out of these clones represented three independent cDNAs derived from a single mRNA transcript (Fig. 1A). Because no transcriptional start site was found in these clones, a nested RACE-PCR was applied to isolate the 5'-end of the putative β7 integrator. In two independent RACE-PCR experiments, a single cDNA fragment was isolated containing an additional 5' sequence. The resulting full-length cDNA contained an ATG start codon in a translation initiation environment (31) and an open reading frame of 1281 nucleotides encoding 427 amino acids. The overall length of the cDNA including the 5'- and 3'-untranslated regions was 1528 base pairs. Considering the addition of a poly(A) tail, the mRNA size is consistent with a major 1.7-kb mRNA transcript detected in Jurkat and Ramos lymphoma cell lines (Fig. 1C). Interaction with the integrin β7 cytoplasmic domain was confirmed with the full-length protein (data not shown).

A search for protein motifs in the deduced amino acid sequence revealed homology of amino acids 238–248 with a repeat motif found in the regulator of chromosomal condensation (RCC1) (32). Homology with a repeat motif from the β subunit of heterotrimeric G proteins was also identified at position 192–206. In addition, five WD repeat motifs as defined by Neer et al. (33) were detected in the protein sequence (Fig. 1B). The β7-integrin interactor isolated by the yeast interaction trap assay therefore represents a member of the WD repeat protein family and was termed WAIT-1 (WD protein associating with integrin cytoplasmic tails-1).

The abbreviations used are: PCR, polymerase chain reaction; RACE, rapid amplification of cDNA ends; GST, glutathione S-transferase; kb, kilobase(s).
Homology search of nucleic acid and protein data bases demonstrated that WAIT-1 represents the human homologue of the murine eed gene that was recently identified by positional cloning (34, 35). WAIT-1 and EED proteins were 100% identical, and the similarity of the cDNA sequences was 92% (Fig. 2). Moreover, wait-1 is also related to the Drosophila esc gene, which was proposed to play role in the repression of hox gene transcription (36). The overall identity of WAIT-1 and ESC was 51% at the amino acid level and 54% at the DNA level (Fig. 2B and data not shown).

Coprecipitation of WAIT-1 with β7-Integrin—To confirm the interaction of WAIT-1 with the integrin β7 cytoplasmic tail, WAIT-1 was expressed as GST fusion protein and used for coprecipitation experiments. GST fused to the C-terminal activation domain of the murine zinc finger transcription factor Egr-1 (GST-Egr-1) served as a negative control. Equal amounts of fusion proteins were bound to glutathione-Sepharose, and lysates of 38-LXSN or 38-LY7 cell lysates. It should be noted that WAIT-1 association with GST-Egr-1 and GST-WAIT-1. The results depicted in Fig. 3 demonstrate that proteins of approximately 90 and 110 kDa corresponding to the precursor and mature forms of β7 were precipitated by GST-WAIT-1 but not GST-Egr-1 from 38-LXSN cell lysates. It should be noted that WAIT-1 interaction with the integrin β7 cytoplasmic domain was stable in highly stringent detergent conditions (1% Triton X-100 and 0.5% SDS). In contrast, specific protein bands were not detectable when lysates of 38-LXSN cells were precipitated with the GST-WAIT-1 matrix (Fig. 3).

To demonstrate the association of WAIT-1 and β7-integrin in vivo, murine α4- and β7-integrin subunits and the WAIT-Fc or control Fc proteins were transiently expressed in 293 cells. The results in Fig. 4A demonstrate that β7-integrin was coprecipitated with WAIT-Fc but not with the control Fc fragment. Reprobing of the filter with antibodies against human IgG revealed similar expression of WAIT-Fc and Fc proteins (Fig. 4A). In addition, Western blot analysis of total cell lysates indicated comparable expression levels of α4 and β7 subunits in 293 cells transfected with either WAIT-Fc or the control Fc construct (Fig. 4B). Together, these data confirm the results of the yeast interaction trap assays and directly demonstrate the association of WAIT-1 with integrin β7 both in vitro and in vivo.

Specificity of WAIT-1 Interaction for Cytoplasmic Tails of β7-Integrins—To determine whether association with WAIT-1 is specific for the integrin β7 chain, cytoplasmic domains of various integrin subunits and unrelated surface receptors were fused to the lexA DNA binding domain and analyzed for interaction with a WAIT-1-B42 prey construct. Expression of the bait proteins in yeast was confirmed by Western blot analysis using a rabbit polyclonal antiserum against lexA (data not shown). The results in Table I demonstrate that, in addition to β7, the cytoplasmic tails of α4 and αE subunits, which both may form heterodimers with β7, interact with WAIT-1. In contrast, the cytoplasmic domains of integrin subunits β1, β2, and αL, and of the structurally unrelated surface receptors CD4 and CD8, did not associate with WAIT-1 in yeast (Table I). Intrinsic ability of the bait proteins to activate reporter genes in yeast was excluded by control assays using the SEC7 domain of cytohesin-1 as a prey construct. Consistent with previous results (27), the SEC7 domain of cytohesin-1 interacted with the cytoplasmic tail of the integrin β2 chain but not with those of other proteins (Table I). Together, these results indicate that WAIT-1 specifically interacts with both the α and β subunit cytoplasmic tails of β7-integrins.

WAIT-1 Interacts with the Membrane-proximal Region of the β7 Cytoplasmic Tail—To determine the binding site of WAIT-1 in the integrin β7 cytoplasmic tail, recombinant PCR was used to generate various deletion and amino acid substitution mutants as fusion proteins with lexA. The primary structure of the mutants was confirmed by nucleic acid sequencing, and expression in yeast was confirmed by Western blot analysis using polyclonal antiserum against lexA (data not shown). Interaction in yeast of β7 cytoplasmic tail mutants was examined with WAIT-1 or the SEC7 domain of cytohesin-1 fused to the B42 transactivator. The results presented in Table II demonstrate that WAIT-1 interacted with the N-terminal fragment of the β7-integrin cytoplasmic domain spanning amino acids 729–751, but not with the C-terminal portion. Further deletional analysis showed that the membrane proximal nine amino acids of the β7 cytoplasmic tail were sufficient to mediate association with WAIT-1 (Table II). In contrast, no interaction was seen with a 14-amino acid segment located C-terminal of the active peptide (Table II). Sequence comparison of integrin β subunits revealed that a tyrosine residue at position 735 is unique to the membrane-proximal region of β7 (Fig. 4). To test a potential role of Tyr-735 for the interaction of WAIT-1 and β7-integrin, Tyr-735 was exchanged for phenylalanine or alanine. As shown in Table II, WAIT-1 efficiently interacted with the Y735F mutant, whereas interaction was completely abrogated by the Y735A mutation. These data therefore demonstrate specific interaction of the human WD repeat protein WAIT-1 with the human integrin β7 cytoplasmic domain as bait, 14 cDNA clones were isolated that represent distinct fragments of WAIT-1. B, the schematic structure of WAIT-1 protein is given, indicating the location of sequence motifs found in WD repeat proteins (filled boxes), β subunits of heterotrimeric G proteins (Gβ), and the regulator of chromosome condensation-1 (RCC1). C, Northern blots of mRNA derived from the human lymphoma cells Jurkat and Ramos were hybridized with a 32P-labeled WAIT-1 cDNA probe. Major transcripts of 1.7 kb are detected in both cell lines.
membrane-proximal portion of the \( \beta_7 \) subunit cytoplasmic tail, with Tyr-735 being of critical importance. Interestingly, tyrosine residues located at a homologous position are present in the \( \alpha \)4 and \( \alpha \)E but not in the \( \alpha \)L cytoplasmic tail that fails to bind WAIT-1 (Fig. 5).

**Distribution of WAIT-1 mRNA in Human Tissues**—Expression of WAIT-1 in various human tissues was analyzed by Northern blotting using the entire coding region of wait-1 as a probe. Except for peripheral blood leukocytes, predominant mRNA transcripts of 1.7 and 2.2 kb and low levels of 2.7- and 3.2-kb mRNA species were detected in all tissues examined (Fig. 6). In peripheral blood leukocytes, however, the 2.7- and
3.2-kb transcripts were predominant, and the 1.7- and 2.2-kb forms were expressed at low levels (Fig. 6). Equal loading of mRNAs from most tissues was demonstrated by rehybridization of the filter with a β-actin probe (Fig. 6). In addition, it should be noted that in the human T lymphoma line Jurkat and in Ramos B lymphoma cells, the 1.7-kb transcript of wait-1 was predominantly expressed (Fig. 1B). These results therefore suggest that expression of distinct WAIT-1 mRNA species may depend on the type of tissue or the activation state of cells.

**DISCUSSION**

Surface receptors of the integrin family are engaged in regulated cell adhesion and ligand-induced signal transduction events with the cytoplasmic tails of α and β subunits being of critical importance. Using a yeast interaction trap system, we have isolated a human WD repeat protein, WAIT-1, that is homologous to the mouse EED and the Drosophila esc proteins and specifically interacts with the cytoplasmic domains of β7, α4, and αE subunits. A tyrosine residue uniquely found in the membrane-proximal segment of the β7 cytoplasmic tail seems to be important for WAIT-1 interaction. Together with a previous report showing that the WD repeat protein RACK1 associates with various integrin β chains (37), the results presented here suggest the existence of a subfamily of integrin-binding WD repeat proteins.

Several interactors of integrin cytoplasmic tails have been discovered, which may either link integrins to the cytoskeleton or may exert regulatory functions for integrin adhesion and signaling. Putative regulatory proteins recently identified include cytobasin-1, p27Hipp, ILK, β3-endoexin, ICAP-1, and RACK1, which interact with various β subunits including β1, β2, β3, β4, and β5 (27, 37–40) as well as CIB, which was reported to associate with the αIIb cytoplasmic tail (41). It is interesting to note, however, that none of the integrin cytoplasmic tail-associated proteins was reported to recognize both α and β subunits. In contrast, we demonstrate that WAIT-1 interacts not only with the β7 cytoplasmic tail but also with those of α4 and αE subunits, which form heterodimers with the β7 chain. These results suggest a distinct structural basis of WAIT-1 association with integrins and are consistent with WAIT-1 binding to β7-integrins being stabilized by interactions with both α and β chain cytoplasmic domain sequences.

Deletion of the entire cytoplasmic domain of the murine β7 subunit results in highly activated ligand binding by the α4β7-integrin (24). In contrast, deletion of a 34-amino acid C-terminal fragment of the β7 cytoplasmic tail abrogated adhesion of 38C13 B lymphoma cells to α4β7-integrin ligands (24). These results indicated that the membrane-proximal segment of the β7 cytoplasmic domain contains a negative regulatory element for α4β7-mediated cell adhesion. In the present study, mutational analysis was used to demonstrate that a short sequence of nine membrane-proximal amino acids in the β7 cytoplasmic tail is sufficient for WAIT-1 interaction. Moreover, a tyrosine residue at position 735 that is unique to the β7 subunit was shown to be important. It is therefore tempting to speculate that association with WAIT-1 may function to prevent or down-regulate ligand recognition of β7-integrins. WAIT-1 binding to β7-integrins may either retain the integrin in a low affinity conformation or prevent binding of integrin-activating proteins.

The murine WAIT-1 homologue EED was proposed to function as a transcriptional regulator of homeobox genes based on developmental abnormalities of mutant mice (35). A similar function has been suggested for the Drosophila esc gene (36). Consistent with a function in gene regulation, the EED protein was found to suppress the activity of a synthetic promoter when fused to the yeast transcription factor Gal4 (42) and to
associate with Enx1/EZH2 polycomb group proteins (34, 43). It is therefore conceivable that WAIT-1 may not only associate with membrane-bound β7-integrins but, under certain conditions, may also localize to the nucleus. The association with integrins and the potential subcellular distribution of WAIT-1 are therefore reminiscent of β3-endonexin and p27NBR proteins, which were reported to interact with the integrin β3 and β4 cytoplasmic tails, respectively, and to be localized partly to the nucleus (40, 44). It will be interesting to determine whether

![Fig. 3. Integrin α4β7 precipitates with recombinant WAIT-1 fusion protein. 38-β7 and control 38-LXSN B lymphoma cells were lysed in buffer containing 1% Triton X-100 and 0.5% SDS, and lysates were incubated with equal amounts of WAIT-1-GST and Egr-1-GST fusion proteins bound to glutathione-conjugated Sepharose. Proteins isolated were separated by nonreducing SDS-polyacrylamide gel electrophoresis and analyzed by Western blotting using monoclonal anti-human IgG.](image)

![Fig. 4. Association of WAIT-1 and α4β7-integrin in vitro. A. 293 cells were transfected with pRK5-α4, pSRA-β7, and the pCDM7 vector encoding either WAIT-Fc or the Fc fragment of human IgG1 for control. Lysates were incubated with protein A-Sepharose, and adsorbed proteins were analyzed by Western blotting with the anti-β7 monoclonal antibody M301. Similar expression of WAIT-Fc and the control Fc fragment was demonstrated by stripping and reprobing of the filter with antibodies against human IgG. B. Expression of comparable levels of integrin α4 and β7 subunits was demonstrated by Western blot analysis of total lysates of triple-transfected 293 cells.](image)

WAIT-1 shuttles between membrane-associated β7-integrins and the nucleus, thereby linking cell adhesion and signal transduction or regulation of gene expression. Consistent with a role of β7-integrins as signaling molecules, it was shown that ligation of α4β7-integrin on B and T lymphocytes induces tyrosine phosphorylation of several substrates including focal adhesion kinase, lek, fyn, and mitogen-activated protein kinases (25, 45).

Northern blot analysis of various human tissues indicated ubiquitous distribution of WAIT-1 mRNA transcripts, whereas β7-integrin expression is largely limited to leukocytes. These observations suggest alternate functions of WAIT-1 that are not related to its interaction with β7-integrin cytoplasmic domains. Involvement of WAIT-1 in multiple cellular functions is also suggested by findings showing that murine EED mutations are embryonic lethal (35), whereas β7 knockout mice are viable (14). Similar observations have been reported for cytohesin-1, which interacts with the cytoplasmic domain of leukocyte β2-integrins. Cytohesin-1 also shows a broad tissue distribution that only partially overlaps with the expression of integrin β2 (27).

Analysis of WAIT-1 tissue distribution revealed multiple mRNA species of 1.7, 2.2, 2.7, and 3.2 kb that hybridize with the WAIT-1 probe. Different WAIT-1-related mRNA species detected in human tissues may indicate the existence of alternatively spliced WAIT-1 variants or a family of WAIT-1-related WD-repeat proteins. In addition, the presence of alternative polyadenylation sites or alternate transcriptional start sites due to regulation of WAIT-1 by distinct promoters may also account for some of the differences in transcript size. In circulating leukocytes, dominant mRNA transcripts of 2.7 and 3.2 kb were observed, whereas in other tissues including spleen transcripts of 1.7 and 2.2 kb predominated. It is therefore conceivable that alternate forms of WAIT-1 are expressed in circulating and tissue-resident leukocytes. Consistent with this possibility, MadCAM-1, the major ligand of α4β7-integrin, is expressed on endothelial cells of mucosal tissues and in the marginal sinus of spleen (46, 47) and is therefore selectively exposed to circulating leukocytes. Moreover, the 2.2-kb mRNA transcript may conform to the recently described 67-kDa form of EED that starts from an unusual GTG initiation codon upstream of the WAIT-1 translational start site and contains an additional WD repeat (42). In contrast to peripheral blood leukocytes, cultured T and B lymphoma cells predominantly express the 1.7-kb WAIT-1 transcript. Integrin α4β7 adhesion-related functions of normal leukocytes and lymphoma cells differ, however, because both cell types bind MadCAM-1 in vitro, but only normal leukocytes efficiently migrate to mucosal

**TABLE I**

| Cytoplasmic tail | Integrator | Interactor |
|-----------------|------------|------------|
| WAIT-1          | β7         | +          |
|                 | β2         | –          |
|                 | β1         | –          |
|                 | α4         | +          |
|                 | αE         | –          |
|                 | αL         | +          |
|                 | CD4        | –          |
|                 | CD8        | –          |

**FIG. 4.** Association of WAIT-1 and α4β7-integrin in vitro. A. 293 cells were transfected with pRK5-α4, pSRA-β7, and the pCDM7 vector encoding either WAIT-Fc or the Fc fragment of human IgG1 for control. Lysates were incubated with protein A-Sepharose, and adsorbed proteins were analyzed by Western blotting with the anti-β7 monoclonal antibody M301. Similar expression of WAIT-Fc and the control Fc fragment was demonstrated by stripping and reprobing of the filter with antibodies against human IgG. B. Expression of comparable levels of integrin α4 and β7 subunits was demonstrated by Western blot analysis of total lysates of triple-transfected 293 cells.
β7-Integrin Interacting WD Repeat Protein

Mapping of the WAIT-1 binding site in the integrin β7 cytosolic tail

Mutants of the integrin β7 cytosolic tail were fused to the lexA DNA binding domain, and interaction with WAIT-1 or the SEC7 domain of cytohesin-1 linked to the transcriptional activator B42 was tested by induction of β-galactosidase activity in yeast. Expression of bait constructs was verified by Western blot analysis of yeast protein extracts using a polyclonal antiserum against lexA. NT, N-terminal segment; CT, C-terminal segment; NT-MP, membrane-proximal region of NT; NT-MD, membrane-distal region of NT.

| Integrin β7 cytosolic tail constructs | WAIT-1 | Cytohesin-1/SEC7 |
|--------------------------------------|--------|-----------------|
| Full-length                           | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| NT                                   | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| CT                                   | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| NT-MP                                | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| NT-MD                                | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| NT-MP Y735F                          | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |
| NT-MP Y735A                          | RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL | + | – |

\[ \begin{align*}
\beta 1 & \text{ KLMIIMHDEFRAFKEFEKMDKQNKWNPYR}\text{SAVTTVTNPKYEGK} \\
\beta 2 & \text{ KALIHLSDLREYRREFKELRKSQGN}}-\text{NDNPLKSATTTNPRFQEDSPTL} \\
\beta 3 & \text{ KLLITIHDREKAFKKEERARAKWD}\text{T3NNPLYKEATSTPTNITYVRTG} \\
\beta 5 & \text{ KLLVTIHDRERAFKQSERSRAYENASNLPRKPISTHTVDFTNKFSNYGTVD} \\
\beta 6 & \text{ KLLV5HFERKEVRKAEASERKAKWQTGTNLRYGSTSTFENVKYHKEQVVDSTD} \\
\beta 7 & \text{ RLSVEIYDREYSRFEEKQQLNLNWQDNSPLYSKAITTTINPRFQEDSPTL} \\
\alpha 4 & \text{ KAGFFKKEQSLQRENKDSWSYINSKGD} \\
\alpha E & \text{ KCGFFKKEQSLQNLIESIRKQLKENLLEEN} \\
\alpha L & \text{ KVGFFKRLKEKMEAGRYVGNIQFAEDSEQLSQGSEADGPCKLPEKDSGSGQKD} \\
\end{align*} \]

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