Purification, Structural Analysis, and Function of Natural ATAC, a Cytokine Secreted by CD8+ T Cells*

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Recently, we identified a novel putative human cytokine expressed by activated CD8+ T cells, which was designated ATAC (activation-induced, T cell-derived, and chemokine-related; the same molecule has been identified independently as lymphotactin and single cysteine motif-1). In this report, we provide evidence that ATAC is a secreted 93-amino acid protein that is generated from its precursor by proteolytic cleavage between Gly23 and Val32. An estimated 60% of ATAC (Val22–Gly114) is secreted as an unmodified protein with a molecular mass of 10,271.72 Da (apparent molecular mass of 12 kDa in SDS-polyacrylamide gel electrophoresis) and in which Cys32 and Cys69 are linked by a disulfide bridge. Unmodified ATAC is a cationic protein with a pI of 11.35 and is capable of binding to heparin. Some 40% of ATAC is O-glycosylated within 25 min of synthesis, giving rise to the appearance of a homogeneous 15-kDa (minor fraction) and a heterogeneous, terminally sialylated 17–19-kDa (major fraction) protein species in SDS-polyacrylamide gel electrophoresis. The secretion of all ATAC protein variants is completed within 30–40 min of synthesis. In terms of function, various ATAC protein forms were consistently ineffective in chemotaxis assays. In contrast, both purified natural ATAC and a chemically synthesized aglycosyl analog induced locomotion (chemokinesis) in purified CD4+ and CD8+ T cell populations at 400 ng/ml.

When screening our collection of 100 human T cell activation genes (1) for “two-signal” genes with expression restricted to T cells, we recently identified a novel cDNA, which was designated ATAC (activation-induced, T cell-derived, and chemokine-related) (2). Induction of ATAC in human T lymphocytes requires simultaneous stimulation by the phorbol ester PMA1 (signal 1) and ionomycin (signal 2) and is suppressed by cyclosporin A (2); it thus resembles the expression characteristics of a number of lymphokine genes (e.g. interleukin-2, interleukin-8, and tumor necrosis factor-α) (3–5). The open reading frame of ATAC encodes a protein of 114 amino acids with an overall structural similarity to chemokines of the CXC and CC families (2). However, several findings suggest that ATAC represents a new class of cytokine: there is only one cysteine at the corresponding protein locus, indicating a tertiary structure different from the known chemokines, and the selective expression of ATAC in CD8+ T cells and CD8+ thymocytes (2, 6, 7) contrasts with the characteristically broad expression pattern of classical chemokines (8). In addition, the location of the ATAC gene on human chromosome 1q23 (2, 7) differs from CXC and CC chemokine genes, which are clustered on chromosomes 4 and 17, respectively (8).

In parallel with our studies, the same molecule was independently identified both in the human (single cysteine motif-1) (9) and murine (lymphotactin) (6) systems. The predicted structural features of the murine molecule and its expression pattern and chromosomal location are analogous to the human counterpart (6). In terms of function, murine and human lymphotactin was reported to be modestly chemotactic on several lymphocyte populations (6, 7). In contrast to these findings, we failed to demonstrate chemotactic effects of ATAC on a variety of cell types with several forms of the putative secreted protein (2).

All groups identified ATAC/single cysteine motif-1/lymphotactin at the cDNA level using methods of reverse genetics, without knowing the biological role of the molecule. The functional experiments reported to date were thus based on the hypothesis that mature ATAC is a secreted molecule and were performed with recombinant proteins assumed to structurally represent the natural cytokine. Since it has been shown for several chemokines that their function is strictly dependent on the correct NH2 terminus (the addition or loss of even a single amino acid residue can have dramatic functional consequences) (10–12) and given the difficulty of exactly predicting the putative signal peptidase cleavage site in the ATAC precursor protein, it was imperative to determine the exact nature of mature ATAC before performing further functional studies. This report describes the purification and structural and biochemical characterization of secreted ATAC. In addition, we provide initial functional data obtained with purified natural ATAC and a synthetic analog.

EXPERIMENTAL PROCEDURES

Expression of ATAC (Gly22–Gly114) in Escherichia coli and Generation of an ATAC-Specific Goat Antiserum—The polymerase chain reaction was used to introduce a SpH I restriction site proximal to Gly22 of human ATAC cDNA and a HindIII restriction site distal to the stop codon of the open reading frame. The resulting SpH I–HindIII fragment was subcloned into the vector pQE (Qiagen Inc.) to express recombinant fusion proteins in E. coli M15 according to the manufacturer’s...
instructions. Denatured recombinant protein was purified on a Ni²⁺-nitrilotriacetic acid-agarose column (QIAGEN Inc.) and used for the generation of a goat antiserum.

**Immunoprecipitation, SDS-PAGE, Immunoblotting, and Carbohydrate Analysis**—ATAC was immunoprecipitated from supernatants of nylon wool-purified lymphocytes or by PMA (20 ng/ml, Sigma) and ionomycin (500 ng/ml, Sigma) for 24 h or from supernatants of transfected 7T. For detection of intracellular ATAC, 5 × 10⁶ cells were lysed in 500 µl of lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1% Nonidet P-40). Quantitative immunoprecipitations were performed using anti-ATAC-specific monoclonal antibody ASA-1 ⁵ ² and the ATAC-specific antisera. In some experiments, brefeldin A (Sigma) was added for the last 4 h of cell stimulation at 0.5 µg/ml. Immunoprecipitated proteins were subjected to SDS-PAGE (13) on 14% gels under reducing conditions (unless otherwise indicated) using a 6–60-kDa ladder (Life Technologies, Inc.) as a molecular mass marker, transferred electrochemically onto a polyvinylidene difluoride membrane (TropiFlour, Tropix Inc.), and stained with goat anti-ATAC serum using the Western Light kit (Tropix Inc.). For carbohydrate analysis, SDS-PAGE-separated proteins were blotted onto nitrocellulose (BA-S 85, Schleicher & Schuell) and stained using the glycan/protein double-labeling system (Boehringer Mannheim, 1210238).

**Purification of Natural ATAC**—CD8⁺ T cells (4–5 × 10⁶) were isolated from nylon wool-treated peripheral blood mononuclear cells using Magnetolabs to a purity of >98% and activated at 2 × 10⁶ cells/ml with PMA and ionomycin for 12–40 h in RPMI 1640 medium supplemented with 5% fetal calf serum. ATAC was purified from the supernatants using an affinity column generated by coupling mAb ASA-1 to HiTrap-Pharose (Pharmacia Biotech Inc.) according to the manufacturer’s instructions. Bound material was eluted with 100 mM glycine, pH 3.0, and further purified by microbore Mono S cation-exchange HPLC followed by reversed-phase RP18 HPLC.

**NH₂-terminal Sequencing and Mass Spectrometry**—An automated Applied Biosystems Procise sequencer was used for NH₂-terminal sequencing of purified ATAC. The matrix-assisted laser desorption ionization mass spectrometer was recorded on a REFLEX mass spectrometer (Bruker, Bremen, Germany) equipped with a Nd₂³⁺ laser in linear mode. Sinapinic acid or o-cyano-4-hydroxycinamic acid was used as the matrix/saturated solution in aqueous 0.1% trifluoroacetic acid/acetonitrile at a ratio of 2:1. The protein was dissolved in aqueous 0.1% trifluoroacetic acid/acetonitrile (1:1) and mixed 1:1 with the matrix solution. The electrospray ionization mass spectrometer was obtained on a TQ 700 quadrupole mass spectrometer (Finnigan Mat, Bremen, Germany) equipped with an electrospray ion source. ATAC was dissolved in methanol/water/acetic acid (50:50:1) and introduced into the ion source by a microsyringe pump (Harvard Apparatus Ltd.) at a flow rate of 0.5 µl/min.

**Establishment of an ATAC Transfectant—**ATAC cDNA containing the entire open reading frame (2) was cloned into the BGMCSneo vector (14) and transfected into myeloma Flx63Ag8.653 cells (American Type Culture Collection) by electroporation. Clone 7T. 0 was selected for high ATAC mRNA expression.

**Chemical Synthesis of ATAC (Val₁⁻⁻⁻⁴-Gly₄⁻⁻⁻⁴)—**ATAC corresponding to the natural protein was synthesized using solid-phase methods, HPLC-purified, renatured, and analyzed by mass spectrometry as described elsewhere (10, 11).

**Chenokinesis Assay**—CD4⁺ and CD8⁺ cells were positively selected (10, 11) using MicroBiomeSelect CD4⁺ or CD8⁺ Magnetolabs coated with mAb CD4⁺ (CD4, IgM, Dynal, Inc.) or ITI-5C2 (CD8, IgM; Dynal, Inc.) at a bead/cell ratio of 3:1 and subsequently detached from the beads (45 min, 20 °C) with polyclonal anti-Fab antibodies (Dynal, Inc.). Purified cell populations were >99% CD3⁺ and 96–99% CD4⁺ or CD8⁺. Contamination with CD14⁺, CD19⁺, CD37⁺, and CD57⁺ cells and nonspecifically isolated CD4⁺ or CD8⁺ cells was <1.5%. Viability of purified T cell populations was verified by trypan blue exclusion and was always >97%. Control experiments excluded an influence of the purification procedure on the migration and phenotype of the analyzed cell populations (15). T cells (3 × 10⁶) were suspended in a collagen solution containing 1.6 mg/ml type I bovine dermal collagen (Vitrogen 100, Collagen Corp., Palo Alto, CA) in Eagle’s modified minimal essential medium, pH 7.4. This suspension was allowed to polymerize (20–30 min, 37 °C, 5% CO₂) in a self-constructed migration chamber. Immediately after polymerization of the lattices, natural or synthetic ATAC was added to the migration chamber. Cell migration was recorded by time-lapse video microscopy at 37 °C (magnification × 64, detection depth >200 mm). Stimulation of T cells with PMA, which induces locomotion in virtually all T cells with little donor variation, was routinely used as a positive control. Locomotor base-line activity and stimulation experiments were performed simultaneously using independent time-lapse units. For evaluation of cell locomotion, the paths of 30 randomly selected cells were digitized as x and y coordinates at a 60-s time interval from step to step using computer-assisted cell tracking as described previously (15, 16). For evaluation of the dose-response experiments, the mean percentage of cells locomoting over the entire observation period in response to phosphate-buffered saline (locomotor base-line activity) was subtracted from the mean percentage of cells locomoting in response to the various concentrations of ATAC dissolved in phosphate-buffered saline.

**RESULTS**

**Natural ATAC Is Secreted by Activated CD8⁺ T Cells**—To determine whether ATAC is a secreted protein, CD8⁺ T cells or nylon wool-purified lymphocytes were stimulated with PMA and ionomycin, and the cell lysates and supernatants were subjected to immunoprecipitation with ATAC-specific mAb ASA-1. The immunoprecipitates were separated by SDS-PAGE and immunoblotted using an antiseraurum generated against a recombinant ATAC protein. Whereas no signal could be detected in lysates and supernatants of unstimulated cells as controls (Fig. 1A, lanes 1 and 2), a faint band of ~12 kDa was observed in lysates of activated lymphocytes (lane 3). With supernatants of activated cells, a major band of ~12 kDa, a sharp band of 15 kDa, and a diffuse band ranging from 17 to 19 kDa were obtained (Fig. 1A, lane 4). When brefeldin A, an inhibitor of intracellular transport and protein secretion (17), was added for the last 4 h of cell culture, a substantial increase in the 12-kDa signal was observed in immunoprecipitates of cell lysates (Fig. 2A, lane 4). These results determined that natural ATAC is a mixture of several protein species with differing molecular masses. To exclude any selectivity of mAb ASA-1 among the forms of natural ATAC, supernatants of activated CD8⁺ T cells were also subjected to immunoprecipitation using an ATAC-specific antisera (Fig. 1B, lane 2; preimmune serum control, lane 1) in parallel with mAb ASA-1 (lane 3).

**Kinetics of ATAC Generation and Secretion**—To determine the kinetics of ATAC generation, nylon wool-purified lymphocytes were activated with PMA and ionomycin for 4 h to achieve maximal ATAC mRNA levels (2). The cells were purified with [³⁵S]Met/Cys for 15 min and chased for various time intervals. Within 25 min of synthesis (15-min pulse, 10-min washing), all ATAC protein species (12, 15, and 17–19 kDa) could be detected in immunoprecipitated cell lysates (Fig. 2A, lanes 3 and 4). All variants of ATAC could also be detected in supernatants of activated lymphocytes as soon as 25 min after synthesis and accumulated thereafter (Fig. 2, lanes 9–13). Lymphocytes activated with PMA (which alone cannot induce the ATAC gene) (2) and the corresponding supernatants were used as specificity controls (Fig. 2, lanes 1 and 2). The analysis of all cell lysates (Fig. 2, lanes 3–8) and supernatants (lanes 9–13) of PMA- and ionomycin-activated lymphocytes revealed that

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ATAC is secreted within ~30–40 min of synthesis.

**Purification of Natural ATAC—CD8+ lymphocytes were stimulated for up to 24 h with PMA and ionomycin, and ATAC was affinity-purified from the culture supernatants with mAb ASA-1 coupled to Sepharose. When the affinity-bound material was subjected to cation-exchange HPLC, the bulk of the 12- and 15-kDa ATAC species was identified in fraction 12 by immunoblotting (Fig. 3A) and further purified to homogeneity (as determined by SDS-PAGE; data not shown) by reversed-phase HPLC (Fig. 3B and Fig. 4A). Natural ATAC could also be purified by reversed-phase HPLC following enrichment on a heparin column (data not shown).

**Natural ATAC Is a 93-Amino Acid Protein (Val22–Gly114) as Determined by NH2-terminal Sequencing and Mass Spectrometry—**Although our initial purification of ATAC was accompanied by a significant loss of the 17–19-kDa species and a relative reduction of the 15-kDa species (Fig. 4A, lane 1), the material was well suited for the characterization of the protein backbone of ATAC since the comparison with a chemically synthesized ATAC protein (Val22–Gly114) (Fig. 4A, lane 2) indicated that the 12-kDa species is a post-translationally unmodified form of ATAC. This comparison also suggested that the 15- and 17–19-kDa variants (see also Figs. 1 and 2) represent post-translational modifications of the 12-kDa ATAC protein species.

**Automated Edman degradation of the purified ATAC preparation shown in Fig. 4A determined Val22 as the NH2-terminal amino acid residue. This result indicated that mature ATAC is generated from the precursor molecule by proteolytic cleavage between Gly21 and Val22.** When the purified ATAC preparation was subjected to matrix-assisted laser desorption ionization mass spectrometry (Fig. 4B) and electrospray mass spectrometry (data not shown), the mass obtained (10,272.1 Da) corresponded well to the calculated 10,271.72 Da for an unmodified Val22–Gly114 ATAC protein. Thus, an estimated 60% of natural ATAC (~12-kDa species, 10,271.72 Da; compare Figs. 1 and 2) is secreted as an unmodified protein of 93 amino acids (Fig. 4C). For reasons given in the legend to Fig. 4, the 15-kDa species present in the preparation did not give a signal in mass spectrometry.

In matrix-assisted laser desorption ionization analysis (Fig. 4B) and electrospray mass spectrometry (data not shown), a smaller second peak with a mass of 10,116.9 Da was observed. Since Edman degradation of two independently purified ATAC preparations did not reveal contaminating proteins or the presence of NH2-terminally truncated ATAC protein variants, this peak possibly resulted from the presence of an ATAC variant lacking the COOH-terminal amino acid residues Thr113 and Gly114 (calculated molecular mass of 10,113.56 Da).

**The 15- and 17–19-kDa Species Are O-Glycosylated Forms of ATAC—**To further characterize the nature of the observed 15- and 17–19-kDa proteins, we transfected myeloma P3x63Ag8.653 cells with a cDNA containing the entire coding region of ATAC (2). When supernatants of transfectant 7.10 were analyzed by immunoprecipitation with mAb ASA-1 followed by immunoblotting, the 12-, 15- and 17–19-kDa protein species identified corresponded to the proteins immunoprecipitated from supernatants of activated lymphocytes (Fig. 5A, compare lanes 1 and 2). No signal was obtained with supernatants of the nontransfected control myeloma cells (data not shown). The 15- and 17–19-kDa protein species could thus be determined as products of the ATAC gene, which arise by post-translational modification of the Val22–Gly114 ATAC protein (12-kDa species). Further analysis demonstrated that both the 15- and 17–19-kDa proteins are glycosylated (Fig. 5B). In the 17–19-kDa protein, the presence of terminal sialic acid α(2–3)-bound to galactose could be detected (Fig. 5C) (a variable degree of glycosylation is apparently responsible for the observed diffuse nature of this ATAC species). Since the ATAC protein sequence does not contain any consensus sites for N-linked glycosylation, the 15- and 17–19-kDa species could thus be identified as O-linked glycosylation forms of mature ATAC.

ATAC Contains a Disulfide Bridge between Cys32 and...
Cys69—Mature ATAC contains only two cysteines, at positions 32 and 69. To test for disulfide bonding between these cysteines, ATAC was immunoprecipitated from supernatants of activated lymphocytes, separated in parallel by SDS-PAGE under reducing (Fig. 6, lanes 1 and 3) and nonreducing (lane 2) conditions, and analyzed by immunoblotting. The altered mobility of the unfolded protein in the reduced state as compared with its mobility in the nonreduced state indicated the presence of a disulfide bond in the mature ATAC protein.

ATAC Induces Locomotion in CD4<sup>+</sup> and CD8<sup>+</sup> Peripheral Blood T Cells—CD4<sup>+</sup> and CD8<sup>+</sup> peripheral blood T cells were purified and tested in a collagen matrix chemokinesis assay for
increased locomotion using time-lapse video microscopy. In all experiments, chemically synthesized ATAC (Val^{22}_{22}–Gly^{114}_{22}) was used at 400 ng/ml (determined to be the optimal concentration after preliminary titrations). In several experiments, preparations of purified natural ATAC containing both the nonglycosylated and glycosylated forms of the cytokine in the correct relative amounts (compare Fig. 1) were used in parallel. With CD4^{+} T cells from different blood donors, a substantial increase in the percentage of migrating cells could be observed in approximately one-third of the experiments (positive results are shown in Fig. 7, A–D). In another one-third of the experiments, there was a modest increase in locomotion; the remaining experiments were negative. The results obtained with purified CD8^{+} T cells were similar. Again, in approximately one-third of the experiments, a clear increase in locomotion was recorded (positive results are shown in Fig. 7, E–H); in another one-third of the experiments, a modest response was observed. When tested in parallel, the effect of natural ATAC was more pronounced when compared with the synthetic ATAC protein (Fig. 7, A and E). The specificity of the chemokinetic effect of natural ATAC on CD4^{+} T cells (Fig. 7C) and CD8^{+} T cells (Fig. 7G) was verified with an antiserum raised against the ATAC protein. Representative dose-response effects of ATAC on CD4^{+} and CD8^{+} T cells are shown in Fig. 7 (D and H). In a number of donors, only one T cell subset responded to ATAC by increased chemokinesis.

**DISCUSSION**

In terms of structure, we have demonstrated in this work that natural ATAC is a secreted protein of 93 amino acids that is generated from the precursor molecule (2, 7, 9) by proteolytic cleavage between Gly^{21} and Val^{22}. Some 60% of ATAC is secreted as an unmodified cationic protein (pI 11.35) with a molecular mass of 10,271.7 Da, running in SDS-PAGE with an apparent molecular mass of 12 kDa. A substantial proportion of ATAC is rapidly glycosylated and secreted within 30–40 min of synthesis; these glycosylated forms of ATAC can be identified by specific labeling of carbohydrate hydroxyl groups using periodate oxidation. The 17–19-kDa ATAC species contains terminal sialic acid α2-3-bound to galactose. The binding of the lectins *Maackia amurensis* agglutinin indicated the presence of terminal sialic acids α2-3-bound to galactose in the 17–19-kDa ATAC species. No binding of the lectins *Sambucus nigra* agglutinin, *Sambucus nigra* agglutinin, *Sambucus nigra* agglutinin, *Galanthus nivalis* agglutinin, and *Datura stramonium* agglutinin was observed with the 15- or 17–19-kDa species (data not shown). NTC, nylon wool-purified T cells.
Biochemical and Functional Characterization of ATAC

FIG. 7

CD4\(^+\) T cells

A

Donor 5

B

Donor 1

C

Donor 4

D

Donor 8

E

CD8\(^+\) T cells

F

Donor 7

G

Donor 10

H

Donor 9

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nat. ATAC

syn. ATAC

nat. ATAC + antiserum

control

ATAC concentration (ng/ml)

ATAC concentration (ng/ml)
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by SDS-PAGE as 15-kDa (minor fraction) and 17–19-kDa (major fraction) proteins. Finally, we could determine that the two cysteines present in the ATAC protein are linked by a disulfide bridge.

Considering the structural aspects, ATAC thus exhibits both similarities to and differences from chemokines of the CC and CXC families, to which it is related by its protein sequence (2, 7–9). It shares with these chemokines a disulfide bridge between the two cysteines conserved in all of these molecules. However, ATAC lacks the other pair of cysteines and the second disulfide bond and thus must have a clearly different tertiary structure compared with the CC and CXC molecules (2, 18). Another conspicuous finding is the comparatively long COOH terminus of ATAC. This finding is relevant, given the observation that modifications of the COOH terminus can be critical for the function of chemokines (10). Unlike CXC chemokines, which are not glycosylated (19), a substantial proportion of ATAC is O-glycosylated and thus resembles MCP-1 (19–21). The functional relevance of the observed glycosylation is unclear at present since the glycosyl form of ATAC is functional in chemokinesis assays with T cells (see below). The cationic nature of the secreted glycosyl form of ATAC and its observed binding to heparin in vitro (data not shown), features shared with the CC and CXC chemokines, potentially enable ATAC to bind to glycosaminoglycans of the tissue matrix and cell membranes and thus to persist in the microenvironment for a prolonged period of time (22, 23).

In terms of function, we could demonstrate that natural ATAC, as well as its nonglycosylated form, can induce substantial chemotaxis in primary CD4+ and CD8+ human T cells. When effective, ATAC induced a similar proportion of T cells to locomote, as did interleukin-8 in our previous experiments (16). For as yet unknown reasons, the effect of ATAC on T cells was clearly donor-dependent (a systematic analysis of this donor dependence will require the identification of the ATAC receptor). More important, when chemically synthesized ATAC was tested in parallel with purified natural ATAC, the results were always concordant. Our findings thus differ from the negative results of the chemokinesis assays reported by Kelner et al. (6), who tested murine lymphotactin for effects on CD4+–depleted, CD8+–depleted, and double-negative murine thymocytes using a filter-based assay system. The interpretation of these divergent results is difficult since both the populations tested and the assay systems used differed considerably; in addition, the assays with murine cells were performed with a form of lymphotactin that probably represents a +1 NH2-terminal variant.

Our numerous attempts to induce chemotaxis with ATAC in various cell systems have remained unsuccessful. We have previously reported negative results obtained with two ATAC molecules, Gly21–Gly114 (+1 NH2-terminal variant) and Gly23–Gly114 (−1 NH2-terminal variant), both generated as fusion proteins with various cell systems have remained unsuccessful. We have on unseparated T cells, purified CD4+ and CD8+ T cells and T cell clones, monocytes, and neutrophils performed earlier with supernatants of the ATAC transfectant 7.10 (compare Fig. 5A) (ATAC at 1 μg/ml to 1 pg/ml) were negative as well (2). New chemotaxis experiments performed with purified natural ATAC (containing all forms of the molecule) on primary human T cells and also T cells prestimulated with phytohemaggutinin and interleukin-2 for 7 days again gave no conclusive results, although the cells were responsive to MCP-1. Thus, our data regarding the chemotactic capabilities of ATAC differ from the studies of Kelner et al. (6) and Kennedy et al. (7), which were performed on murine and human cells using murine (presumed +1 NH2-terminal variant) and human lymphotactin, respectively. The reasons for this discrepancy are unclear at present since the test systems used by these investigators were similar to ours. Further experiments will be necessary to determine whether ATAC can functionally be grouped into the family of chemokines.

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Fig. 7. Locomotion of CD4+ and CD8+ T cells in response to natural and synthetic ATAC proteins. Purified CD4+ and CD8+ T cells were suspended in a collagen matrix, and natural (nat.) or synthetic (syn.) ATAC (Val22–Gly114) was added at 400 ng/ml. Control experiments were set in parallel. Time-dependent changes in T cell locomotion were recorded by time-lapse video microscopy. Experiments with CD4+ and CD8+ T cells are shown in A–D and E–H, respectively. A direct comparison of the effects of natural and synthetic ATAC proteins is shown in A and E. The chemokinetic effect of purified natural ATAC on CD4+ T cells (C) and CD8+ T cells (G) was inhibited by an ATAC-specific antiserum (1:100 dilution), but not by a preimmune serum at the same dilution (data not shown). Dose–response effects of synthetic ATAC (Val22–Gly114) were tested on CD4+ and CD8+ T cells of three responders; representative results are shown in D and H. Locomotor base-line activity in response to an equivalent volume of phosphate-buffered saline (solvent for ATAC) is indicated in all experiments (control).