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Immunology

Attractin (DPPT-L), a member of the CUB family of cell adhesion and guidance proteins, is secreted by activated human T lymphocytes and modulates immune cell interactions

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ABSTRACT **Attractin is a normal human serum glycoprotein of 175 kDa that is rapidly expressed on activated T cells and released extracellularly after 48–72 hr. We have cloned attractin and find that, as in its natural serum form, it mediates the spreading of monocytes that become the focus for the clustering of nonproliferating T lymphocytes. There are two mRNA species with hematopoietic tissue-specific expression that code for a 134-kDa protein with a putative serine protease catalytic serine, four EGF-like motifs, a CUB domain, a C type lectin domain, and a domain homologous with the ligand-binding region of the common γ cytokine chain. Except for the latter two domains, the overall structure shares high homology with the *Caenorhabditis elegans* F33C8.1 protein, suggesting that attractin has evolved new domains and functions in parallel with the development of cell-mediated immunity.**

Analysis of the immune response *in vitro* allows basic interactions between cells and soluble modulators to be studied, but interpretations are difficult to extend to actual responses *in vivo* where reactions occur in complex cellular environments with constant dynamic modification of the extracellular environment. An important role is played by the extracellular matrix, which interacts with adhesion structures on the surface of immune cells, directing cell migration, localization, and clustering, after which it influences the activity of local cytokines and lymphokines (1, 2). The passage of activated leukocytes between endothelial cells and their migration through the extracellular matrix to sites of inflammation is facilitated by the up-regulated surface expression of several adhesion molecules and proteases (3).

On activated T cells, one of the most prominently expressed proteases is CD26, which is a marker of T lymphocytes capable of migrating across endothelial barriers (4, 5) and has a collagen-binding domain (6). CD26 is now known to be identical with both dipeptidyl peptidase IV (DPPIV; ref. 7) and adenosine deaminase binding protein (8). The multifunctionality of CD26, which is the prototype for a family of related molecules that include fibroblast activation protein-α (9), DPPVI (10), and Seprase (11, 12), has expanded to include T lymphocyte costimulatory activity, enhancing immune responses channeled through the CD3/T cell-receptor complex (13).

Previously, we had found a soluble serum form of DPPIV (14), and its circulating levels were related to the ability of peripheral blood mononuclear cells (PBMC) to react *in vitro* to recall antigens such as tetanus toxoid. We purified the serum DPPIV/CD26 and characterized it as a 175-kDa glycoprotein with DPPIV activity that we termed DPPT-L, because it was distinct from 105-kDa DPPIV/CD26 (15). DPPT-L displayed some CD26 antigenic epitopes, was rapidly expressed as a T lymphocyte activation antigen (16), was released from the lymphocyte membrane after 48–72 hr, and could up-regulate recall antigen-specific T cell responses in a manner similar to that of CD26.

In this report we describe the spreading of monocytes and clustering of T cells induced by both purified serum and recombinant DPPT-L. There is no significant sequence homology between DPPT-L and CD26 or any other characterized human protein. To reflect the lack of sequence homology with DPPIV/CD26, and to take into account its biological activity, we have changed the name from DPPT-L to attractin. We propose that the T lymphocyte uses attractin to marshal together the cells required to form a cluster of cooperating immune cells.

EXPERIMENTAL PROCEDURES

Cell Techniques. PBMC, E^+ T cells, and E^- monocytes/B cells were purified as described (17). CHO ($dhfr^-$) cells and the Jurkat T cell line were obtained from the American Type Culture Collection. 293T cells were obtained from B. Mayer (Children's Hospital, Boston, MA). All cell lines were maintained in RPMI medium 1640 containing 10% fetal bovine serum. To assess the biological effects of attractin, leukocytes were cultured in serum-free AIM V medium (Life Technologies, Gaithersburg, MD) in 48-well plates (Costar). For cell activation, E^+ lymphocytes (10^6 per ml) supplemented with 0.1% E^- cells were incubated in AIM V medium together with phytohemagglutinin (PHA, 1 μg/ml; Murex Diagnostics, Dartford, U.K.) for 48 hr. Cell proliferation was assessed by using ^{3}H-labeled thymidine incorporation as described (15).

RNA/DNA Preparation and Analysis. mRNA was isolated by using the Poly(A)Pure kit (Ambion, Austin, TX). Northern blots were prepared by using standard denaturing formaldehyde agarose electrophoresis techniques and transferred to GeneScreen Plus (DuPont/NEN). The EST clone R84298 was obtained from the I.M.A.G.E. consortium (18) through the American Type Culture Collection. Both fetal liver cDNA libraries (λgt11 and Marathon cDNA) were obtained from CLONTECH. The JSDC T cell library was prepared from 48-hr PHA-activated T lymphocyte mRNA with the Superscript Choice system (Life Technologies) and ligated into

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Abbreviations: PHA, phytohemagglutinin; PBMC, peripheral blood mononuclear cells.

Data deposition: The nucleotide sequence coding for attractin reported in this paper has been deposited in the GenBank database (accession no. AF034957).

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pcDNAI/Amp (Invitrogen). The GF activated T cell library was prepared and ligated into pCDM8 as described (18). The expression vectors pRc/CMV and pSecTag2B were obtained from Invitrogen. All DNA probes were labeled with [^{32}P]dCTP by random priming (Life Technologies).

Isolation of cDNA Encoding Attractin. Tryptic/chymotryptic peptides were prepared and analyzed as described (15). A multiple human hematopoietic tissue Northern blot (CLONTECH) was screened with the 1.2-kb *Mfe*I–*Eco*NI R84298 fragment. The λgt11-fetal liver library was screened with the 1.3-kb *Cla*I–*Hin*dIII R84298 fragment that yielded pks-43 (4 kb). *Hin*cIII digestion of pks-43 released a 5′ 982-bp fragment, which was used to rescreen the fetal liver library, and a further 5′ sequence including the putative start codon was identified (pks-43-1). Full-length attractin was made by ligating the *Hin*dIII–*Nae*I digest of the PCR-amplified pks-43-1 fragment (CCCAAGCTTGGGATGGGTGTCGGGCTCAGCCGC-forward, ATAAGAATGCGGCCGCTAAACTCATTGT-TCAGTTTGCACCTG-reverse) with the *Nae*I–*Not*I digest of the PCR-amplified pks-43 fragment (CCCAAGCTTGGGA-TGGTGGCCGCAGCGGCGGC-forward, CCAGGTCCAT-CTGTCACAAACCCAG-reverse) together with *Hin*dIII–*Not*I-digested pRc/CMV. For cloning of attractin with disabled start and stop codons into pSecTag2B, a 3.5-kb fragment was amplified from pRc/CMV-attractin with the Advantage GC cDNA PCR system (CLONTECH) and the primers GTG-CGTGAAGCTTGTACCGGCAACTGAGGCAAGGCTG-A-forward and GTAGTTTTAAGTCCACGTTTGACTTC-GCCGGCGTGCGTG-reverse, digested with *Hin*dIII–*Not*I, and ligated into pSecTag2B.

Expression of Functional Attractin. For *in vitro* transcription and translation, the Quick TnT system together with canine microsomal membranes was used (Promega). 293T cells were transfected transiently with pSecTag2B-attractin complexed with Lipofectamine Plus (Life Technologies) and assayed for attractin expression at 48 hr. For Western blotting experiments, cells were lysed in boiling SDS/PAGE sample buffer (2×). Samples were run on SDS/PAGE gels and transferred to nitrocellulose by electroblotting. The membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 1% BSA. Blots were incubated with murine anti-myc (1:5000, Invitrogen) followed by anti-mouse IgG-HRP (1:5000, Amersham) or with monoclonal anti-myc-HRP (1:2000, Invitrogen) and detected with the Phototope chemiluminescent system (New England Biolabs). For immunoprecipitation experiments, the cells were solubilized in lysis buffer (1% Triton X-100/0.1% Nonidet P-40/150 mM NaCl). Lysates were precleared with mIgG-agarose beads (Sigma) followed by incubation with protein A-purified polyclonal rabbit anti-attractin or protein A-purified normal preimmune IgG. Antibody complexes were isolated by incubation with anti-rabbit IgG-agarose beads (Sigma) followed by boiling in 2× SDS/PAGE loading buffer. Thereafter, the procedure was identical with that described for Western transfers above.

For stable transfections, pSecTag2B-attractin was introduced into CHO cells by electroporation (250 V, 1600 μF) with the Cell-Porator apparatus (Life Technologies) and selection with zeocin (500 μg/ml, Invitrogen). To purify recombinant attractin, cells were lysed and loaded onto a Talon Superflow metal affinity resin (CLONTECH) and eluted with 250 mM imidazole. DPPIV activity of the recombinant attractin was determined by using Gly-Pro-pNA as substrate as described (15).

Binding Assays. PBMC were activated for 24 hr with PHA in AIM V medium as described above and washed in AIM V. Thereafter, PBMC (10^6 per 100 μl) were incubated for 1 hr at 4°C with doubling dilutions of ^{125}I-labeled attractin (16), starting at 2 μg/ml (10^7 dpm/μg). The cells were washed with cold PBS, and the pellet and first wash supernatant were counted by γ scintillation. Results were analyzed by Scatchard analysis.

Electron Microscopy. Cells were prepared as described (20) and were analyzed by transmission electron microscopy (model JEM 100 CX II; JEOL).

RESULTS

Previously, we found that purified-serum attractin could enhance the proliferative responses of PBMC to recall antigens such as tetanus toxoid. In the absence of the antigen, the attractin had no effect (15). We determined whether attractin was binding directly to leukocytes and found by Scatchard analysis that there were ≈1000 bound molecules of attractin/resting T cell, with ≈2000 bound molecules/PHA-activated T cell, with a K_d for both between 5 and 50 pM, indicative of a specific high affinity interaction (data not shown). In determining whether the binding of attractin had a functional effect on cells, we found that within 48 hr of its addition to PBMC in serum-free medium, attractin caused a spreading of adherent macrophage-like cells that generated long processes to which lymphocytes attached. This process was dose dependent, with the maximum effect occurring in doses between 5 and 10 μg/ml attractin (Fig. 1 *A–F*). Upon incubation with E^+ T lymphocytes or E^- monocytes/B lymphocytes alone, attractin had no effect, but clustering occurred if the E^- and E^+ populations were recombined (Fig. 1 *G–I*). After washing away nonadherent cells, the adherent cells were released by incubation with EDTA in PBS. By using immunofluorescence analysis, we found that the small adherent cells were exclusively $CD3^+$ T cells, whereas the large adherent cells were predominantly $CD14^+$ monocyte/macrophages.

To demonstrate the specificity of the spreading-clustering effect, we required recombinant attractin and began isolating a full-length cDNA clone by identifying specific peptide sequences. Attractin was purified to homogeneity, and the N termini of 16 proteolytic peptides were sequenced (underlined in Fig. 2). One of the sequences (17 amino acids) matched 100% with a translated 3′ EST sequence (R84298) that coded for a 1.9-kb sequence including the 3′ end of attractin. By using this sequence as a probe, we observed two mRNA species of 4.4 kb and 8–9 kb, both of which were represented heavily in fetal liver and spleen (Fig. 3*A*). The larger form was dominant in thymus, whereas the lower form was dominant in PBMC. No up-regulation of attractin mRNA transcription was observed after T cells were activated (Fig. 3*B*).

A fetal liver library was screened based on mRNA expression, and a 982 bp 5′ fragment derived from the longest clone (pks-43) was used to rescreen the library leading to identification of another clone with an extra 5′ sequence (pks-43-1). Sequencing of both overlapping clones yielded an ORF of 3.594 kb that encoded all 16 peptides identified (Fig. 2). PCR amplification of the main body of attractin cDNA from the two activated T cell libraries and the fetal liver library produced an identical 3-kb PCR product (Fig. 3*C*). The first methionine is within a consensus Kozak sequence, and the subsequent ORF codes for a 134-kDa protein with 26 potential N-glycosylation sites. Although attractin is glycosylated heavily (15), we could not identify a consensus leader sequence-signal peptide. Likewise, despite membrane expression on 48-hr activated T cells (16), no transmembrane sequence could be predicted. Several distinct domains and motifs could be identified in the ORF, as depicted in Fig. 4*B*. These include a serine (Ser-26) within a hybrid of the prolyl oligopeptidase and trypsin-like serine protease catalytic motifs (Fig. 4*C*), an EGF domain (Gly-24 to Gln-54), a CUB domain (His-57 to Phe-173), an EGF domain (Met-175 to Ala-207), the ligand-binding motif of the common γ cytokine chain (Cys-636 to Trp-648), a C type lectin domain (Ile-713 to Cys-844), and 2 cysteine-rich regions incorporating

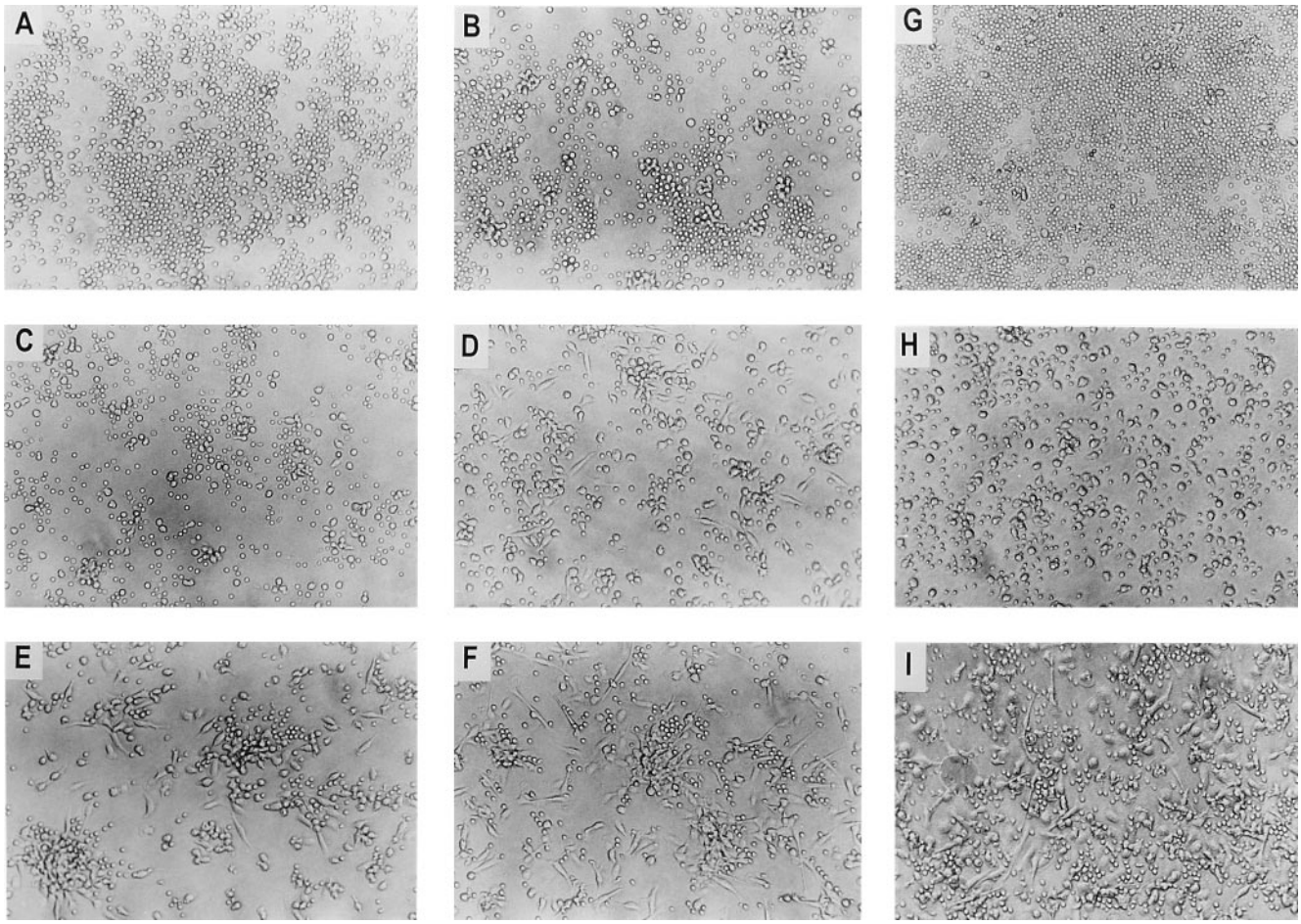

FIG. 1. Purified natural attractin mediates monocyte spreading and T cell clustering after 48 hr. (*A*) Control, no addition; (*B*) 0.5 μg/ml; (*C*) 1 μg/ml; (*D*) 2 μg/ml; (*E*) 5 μg/ml; (*F*) 10 μg/ml; Attractin at 10 μg/ml was added for 48 hr to PBMC, separated into E^+ T lymphocytes (*G*), E^- monocytes/B cells (*H*), and to T lymphocytes remixed with monocytes/B cells (*I*).

the C-terminal laminin-like EGF domains (Ala-988 to Lys-1031 and Pro-1034 to Cys-1066).

The only highly significant match at both the nucleotide and amino acid level (31% identity, 45% similarity across the complete 1198 amino acids) is with the nematode F33C8.1 perlecan-like protein, which has an identical organization of the CUB and EGF domains and a conserved positioning of cysteines, suggesting a similar secondary structure (Fig. 4*B*). In contrast to attractin, F33C8.1 does not express the γ chain binding motif or the C type lectin domain.

To understand the secretory route followed by attractin in the absence of a signal peptide, we determined the subcellular localization of attractin in resting and activated E^+ T lymphocytes. No glycosylated attractin could be detected anywhere in resting T cells (Fig. 5*A*), whereas in T cells activated for 48 hr with PHA, attractin was localized clearly in large vesicular structures (Fig. 5*B*) that often contained an electron-dense core (Fig. 5*C*). Vesicles containing attractin were often clustered close to the plasma membrane where they released attractin into the extracellular space (Fig. 5*D*). Attractin was cloned into the expression vector pRc/CMV, but glycosylated attractin could not be detected in transfected Chinese hamster ovary (CHO) cells. We forced the posttranslational glycosylation of attractin by cloning attractin into pSecTag2B which supplies an N-terminal leader sequence. In an *in vitro* transcription/translation system, pSecTag2B-attractin coded for a protein of 134 kDa and yielded a product of about 180 kDa in the presence of glycosyl transferases (Fig. 6*A*). The correctly sized product was also detected by Western blotting with anti-myc of a whole cell lysate of 293T cells transiently transfected with pSecTag2B-attractin (Fig. 6*B*). The polyclonal antiattractin could immunoprecipitate recombinant attractin from CHO cells stably transfected with pSecTag2B-attractin confirming that the overall structure of the recombinant protein was similar to that of the purified natural material (Fig. 6*C*).

Attractin was isolated from lysates of stably transfected CHO cells, because even with a signal peptide, the recombinant attractin localized intracellularly and was not secreted. The DPPIV enzyme activity of the recombinant protein was 0.42 units per mg, in comparison with 0.79 units per mg for T cell-released attractin, 1.78 units per mg for serum attractin, and 4.12 units per mg for recombinant CD26. Because we purify attractin with nondenaturing techniques, there exists the possibility that soluble CD26 binds to the purified attractin and is carried through the purification process. Purified attractin, however, labels with [^{3}H]diisopropyl fluorophosphate (15), which binds to the catalytic serine of serine proteases. Furthermore, we found that the recombinant attractin could be immunoprecipitated by some, but not all, anti-CD26 antibodies (data not shown).

We repeated the PBMC interaction assays depicted in Fig. 1 by using recombinant rather than natural serum-purified attractin. The spreading effect of recombinant attractin was also dose dependent (Fig. 7 *A–D*). At 5 μg/ml, the effect was similar to that of 5 μg/ml natural attractin and confirmed the results observed with purified natural attractin. There was a greater tendency for clustering when the recombinant attractin was used, but these clusters were not proliferating cells, as indicated by the fact that no increase in thymidine incorporation could be detected over background (data not shown).

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   1 MVAAAAATEA RLRRRTAATA ALAGRSGGPH CVNGGRCNPG TGQCVCPAGW
  51 VGEQCQHCGG RFRLTGSSGF VTDGPGNYKY KTKCTWLIEG QPNRIMRLRF
 101 NHFATECSWD HLYVYDGDSI YAPLVAAFSG LIVPERDGNE TVPEVVATSG
 151 YALLHFFSDA AYNLTGFNIT YSFDMCPNNC SGRGECKISN SSETVECECS
 201 ENWKGEACDI PHCTDNCGFP HRGICNSSDV RGCSCFSDWQ GPGCSVPVPA
 251 NQSFWTREEY SNLKLPRASH KAVVNGNIMW VVGGYMFNHS DYNMVLAYDL
 301 ASREWLPLNR SVNNVVVRYG HSLALYKDKI YMYGGKIDPT GNVTNELRVF
 351 HIHNESWVLL TPKAKEQYAV VGHSAHIVTL KNGRVVMLVI FGHCPLYGYI
 401 SNVQEYDLDK NTWSILHTQG ALVQGGYGHS SVYDHRTRAL YVHGGYKAFS
 451 ANKYRLADDL YRYDVDTQMW TILKDSRFFR YLHTAVIVSG TMLVFGGNTH
 501 NDTSMSHGAK CFSSDFMAYD IACDRWSVLP RPDLHHDVNR FGHSAVLHNS
 551 TMYVFGGFNS LLLSDILVFT SEQCDAHRSE AACLAAGPGI RCVWNTGSSQ
 601 CISWALATDE QEEKLKSECF SKRTLDHDRC DQHTDCYSCT ANTNDCHWCN
 651 DHCVPRNHSC SEGQISIFRY ENCPKDNPMY YCNKKTSCRS CALDQNCQWE
 701 PRNQECIALP ENICGIGWHL VGNSCLKITT AKENYDNAKL FCRNHNALLA
 751 SLTTQKKVEF VLKQLRIMQS SQSMSKLTLT PWVGLRKINV SYWCWEDMSP
 801 FTNSLLQWMP SEPSDAGFCG ILSEPSTRGL KAATCINPLN GSVCERPANH
 851 SAKQCRTPCA LRTACGDCTS GSSECMWCSN MKQCVDSNAY VASFPFGQCM
 901 EWYTMSTCPP ENCSGYCTCS HCLEQPGCGW CTDPSNTGKG KCIEGSYKGP
 951 VKMPSQAPTG NFYPQPLLNS SMCLEDSRYN WSFIHCPACQ CNGHSKCINQ
1001 SICEKCENLT TGKHCETCIS GFYGDPTNGG KCQPCKCNGH ASLCNTNTGK
1051 CFCTTKGVKG DECQLCEVEN RYQGNPLRGT CYYTLLIDYQ FTFSLSQEDD
1101 RYYTAINFVA TPDEQNRDLD MFINASKNFN LNITWAASFS AGTQAGEEMP
1151 VVSKTNIKEY KDSFSNEKFD FRNHPNITFF VYVSNFTWPI KIQVQTEQ
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FIG. 2. Peptide sequence of attractin. The sequences identified by N-terminal sequencing of tryptic and chymotryptic peptides are underlined.

DISCUSSION

In this report we describe how attractin mediates an interaction between T lymphocytes and monocytes that leads to the adherence and spreading of monocytes that become foci for T lymphocyte clustering. We cloned attractin, expressed a recombinant form, and showed that the recombinant form has the same functional effect as the natural form. We show that attractin is a new member of the CUB domain family, which includes the complement proteins C1r/C1s, Uegf, and BMP-1 (21). Moreover, by using recombinant attractin, we confirmed the DPPIV activity and crossreactivity with anti-CD26 antibodies that led us to believe that attractin is related to CD26/DPPIV (15, 19).

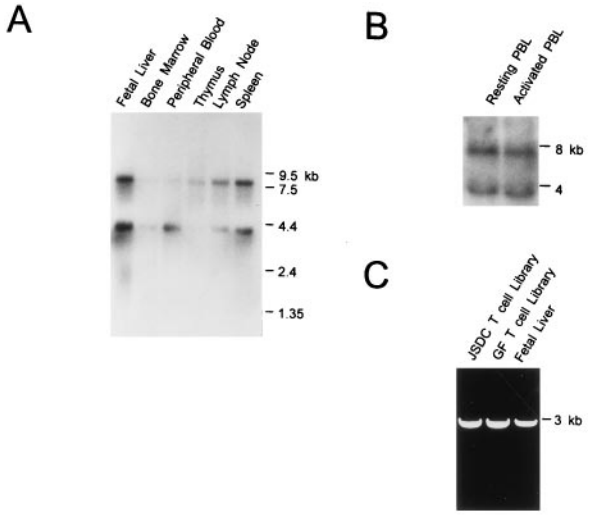

FIG. 3. Attractin mRNA expression. (*A*) Multiple tissue mRNA Northern blot. (*B*) Resting and PHA-activated PBMC total RNA. (*C*) PCR amplification of a 3164-bp fragment (from 3594 bp) of attractin from three independent cDNA libraries.

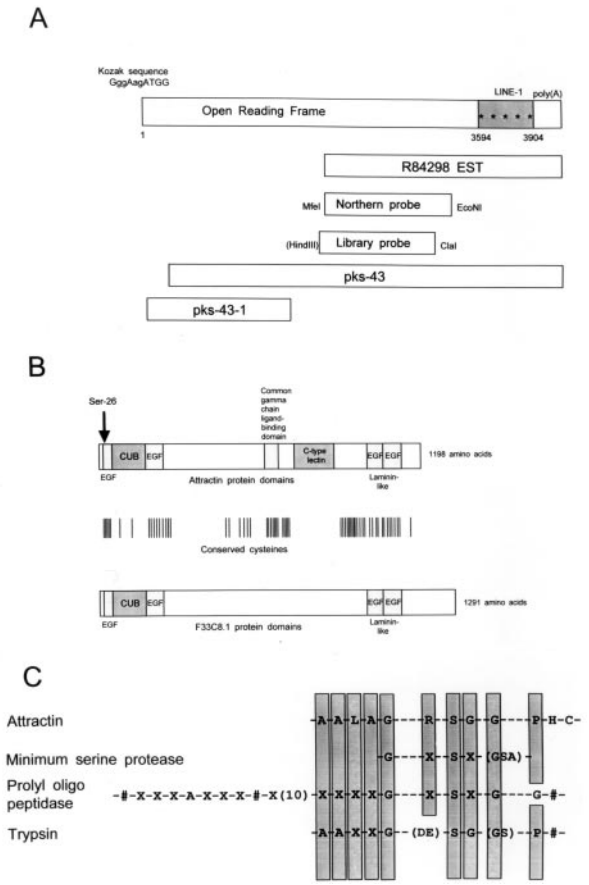

FIG. 4. Organization of attractin cDNA and peptide domains. (*A*) Attractin cDNA: the uppercase bases at the origin represent bases satisfying the Kozak consensus. The asterisks indicate in-frame stop codons. (*B*) Comparison of attractin protein domains and motifs with *C. elegans* F33C8.1 protein, the horizontal bars depict the position of cysteines shared by both sequences. (*C*) Comparison of the putative catalytic serine motif of attractin with the catalytic serine motifs of other serine proteases. The shaded box indicates agreement with the consensus, the # or exclusion from the shaded boxes indicates conflict, and the X indicates satisfaction by any amino acid. The parentheses enclose amino acids any of which would satisfy the consensus.

No difference was found in attractin mRNA expression between resting and activated PBMC, implying a regulatory step between transcription and glycosylation rather than induction of *de novo* mRNA synthesis. Despite extensive N glycosylation of the isolated serum attractin, there is no consensus signal peptide in the cDNA sequence. Although another protein may chaperone attractin through the Golgi complex and endoplasmic reticulum, several proteins are known to be secreted without a signal peptide, including fibroblast growth factor 9 (FGF-9; ref. 22), interleukin 1α (IL-1α) and IL-1β (23), FGF-1 (24), FGF-2 (25), and platelet-derived endothelial cell growth factor (26). This has led to the proposal of alternative secretory pathways with slow exocytic release from large cytoplasmic pools (27). The electron-microscopy results confirm that the early activated T lymphocyte expression results from vesicular release at the plasma membrane. Preliminary results we have obtained exclude GPI-anchoring of attractin, and having activated T cells in serum-free media cleared of attractin, we are certain that it is

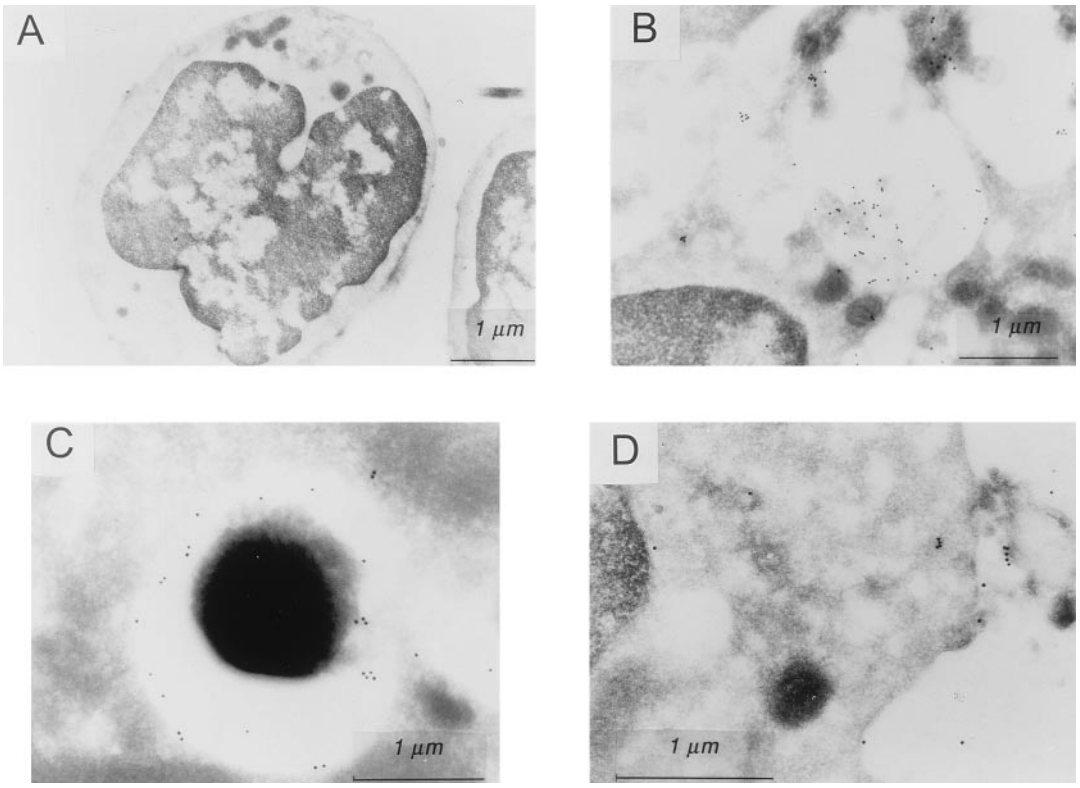

FIG. 5. Intracellular localization of glycosylated attractin. Immunogold electron microscopy using rabbit polyclonal antiattractin. (*A*) Resting T lymphocyte with no evidence of attractin expression. (*B*) In T cells activated for 48 hr with PHA, attractin is expressed in large vacuoles. (*C*) Attractin often localizes in vesicles with electron dense core. (*D*) Vesicles containing attractin break open at the cell surface releasing attractin.

newly synthesized and not acquired from the external environment.

Usually, proteins containing EGF-like motifs are involved in extracellular signaling or cell guidance (28). Attractin also contains a motif representing the ligand-binding region of the cytokine receptor common γ chain (29). We have been unable to identify any functional interaction between attractin and IL-2, IL-4, IL-7, or IL-15, whose receptors include the common γ chain (data not shown). In overall structure and organization of domains, attractin most closely resembles the CUB-containing proteins BMP1 (procollagen C proteinase) that influences cell interactions during development (30). The C type lectin domain recognizes carbohydrate and is characteristic of the Selectin family of proteins involved in the adhesion of leukocytes to vascular endothelia. This domain is also characteristic of proteins involved in endocytosis for antigen processing in macrophages and dendritic cells (31).

There is strong identity between attractin and the 143-kDa F33C8.1 *Caenorhabditis elegans* protein. The potential γ chain ligand binding motif and C type lectin domain present in attractin are missing in the *C. elegans* transcript, suggesting an evolutionary development in which the human form incorporated these new domains in parallel with the development of cell-mediated immunity. Furthermore, the domain structure of the nematode protein characterizes it as a potential extracellular glycoprotein, but there is no evidence of a N-terminal signal sequence.

We had shown earlier that attractin, similar to CD26, is unable to induce cell proliferation in its own right, but is able

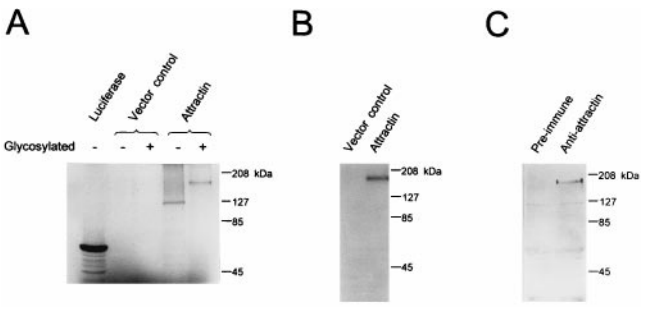

FIG. 6. Expression of recombinant attractin and immunoprecipitation by antibody against natural attractin. (*A*) *In vitro* transcription/translation of attractin in absence or presence of glycosyl transferases. (*B*) Western blotting with anti-myc of lysates of 293T cells transiently transfected with pSecTag2B-attractin or pSecTag2B vector control. (*C*) Attractin was precipitated with preimmune antibody or antiattractin antibody from lysates of CHO cells stably transfected with pSecTag2B-attractin.

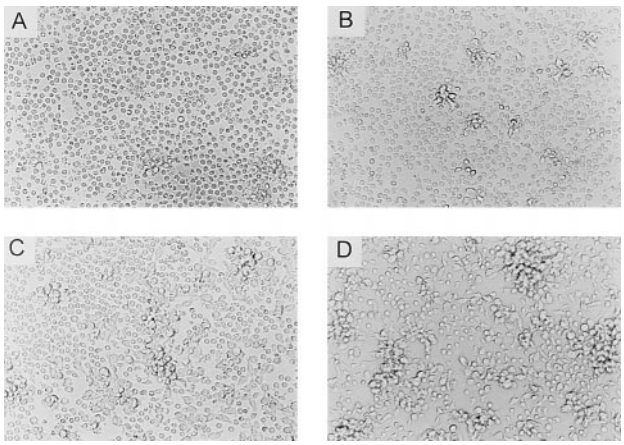

FIG. 7. Recombinant attractin mediates monocyte/macrophage spreading and T cell clustering. Resting PBMC were incubated with increasing concentrations of purified recombinant attractin for 48 hr. (*A*) Control, no addition; (*B*) 1 μg/ml; (*C*) 2 μg/ml; (*D*) 5 μg/ml.

to enhance the proliferative response of PBMC to recall antigens such as tetanus toxoid (15, 16). We propose that attractin may modulate the interaction between T cells and macrophages, allowing a more rapid or more effective means of presenting antigen. It is highly likely that the minimal immunoregulatory unit consists of an antigen-presenting cell that acts as a focus for a cluster of T helper cells and effector cells (32). The association of the three cell types is neither simultaneous nor random; rather, the antigen-presenting cell clusters first with the helper T cells, and this cluster acts as a focus for recognition by effector cells (33). In the absence of antigen, no proliferation occurs in attractin-induced clusters of monocytes and T cells. However, we propose that if a recall antigen such as tetanus toxoid is present, the clustering of cells maximizes the potential response to the antigen. Attractin may regulate local cytokine activity either by influencing binding and presentation or by proteolytic modification. Recently, DPPIV/CD26 has been shown to cleave an N-terminal dipeptide that converts the full-length chemokine RANTES [1–68] from a potent monocyte chemoattractant to RANTES [3–68], an equally potent inhibitor of monocyte chemotaxis (34). The concatenation in attractin of domains related to regulation of cell interactions together with domains related to lymphokine/cytokine binding, the rapid up-regulation of attractin expression by activated T cells, and the clear effect on T cell-monocyte/macrophage association suggest that attractin, a normally circulating serum protein, plays a significant role in the immune response *in vivo*.

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1. Shimizu, Y. & Shaw, S. (1991) *FASEB J.* **5,** 2292–2299.
2. Gilat, D., Cahalon, L., Hershkoviz, R. & Lider, O. (1996) *Immunol. Today* **17,** 16–20.
3. Hauzenberger, D., Klominek, J., Bergstrom, S. E. & Sundqvist, K. G. (1995) *Crit. Rev. Immunol.* **15,** 285–316.
4. Masuyama, J., Berman, J. S., Cruikshank, W. W., Morimoto, C. & Center, D. M. (1992) *J. Immunol.* **148,** 1367–1374.
5. Brezinschek, R. I., Lipsky, P. E., Galea, P., Vita, R. & Oppenheimer-Marks, N. (1995) *J. Immunol.* **154,** 3062–3077.
6. Loster, K., Zeilinger, K., Schuppan, D. & Reutter, W. (1995) *Biochem. Biophys. Res. Commun.* **217,** 341–348.
7. Hegen, M., Niedobitek, G., Klein, C. E., Stein, H. & Fleischer, B. (1990) *J. Immunol.* **144,** 2908–2914.
8. Kameoka, J., Tanaka, T., Nojima, Y., Schlossman, S. F. & Morimoto, C. (1993) *Science* **261,** 466–469.
9. Scanlan, M. J., Raj, B. K., Calvo, B., Garin-Chesa, P., Sanz-Moncasi, M. P., Healey, J. H., Old, L. J. & Rettig, W. J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5657–5661.
10. Wada, K., Yokotani, N., Hunter, C., Doi, K., Wenthold, R. J. & Shimasaki, S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 197–201.
11. Goldstein, L. A., Ghersi, G., Pineiro-Sanchez, M. L., Salamone, M., Yeh, Y., Flessate, D. & Chen, W. T. (1997) *Biochim. Biophys. Acta* **1361,** 11–19.
12. Pineiro-Sanchez, M. L., Goldstein, L. A., Dodt, J., Howard, L., Yeh, Y. & Chen, W. T. (1997) *J. Biol. Chem.* **272,** 7595–7601.
13. Dang, N. H., Torimoto, Y., Deusch, K., Schlossman, S. F. & Morimoto, C. (1990) *J. Immunol.* **144,** 4092–4100.
14. Tanaka, T., Duke-Cohan, J. S., Kameoka, J., Yaron, A., Lee, I., Schlossman, S. F. & Morimoto, C. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 3082–3086.
15. Duke-Cohan, J. S., Morimoto, C., Rocker, J. A. & Schlossman, S. F. (1995) *J. Biol. Chem.* **270,** 14107–14114.
16. Duke-Cohan, J. S., Morimoto, C., Rocker, J. A. & Schlossman, S. F. (1996) *J. Immunol.* **156,** 1714–1721.
17. Morimoto, C., Letvin, N. L., Boyd, A. W., Hagan, M., Brown, H. M., Kornacki, M. M. & Schlossman, S. F. (1985) *J. Immunol.* **134,** 3762–3769.
18. Lennon, G., Auffray, C., Polymeropoulos, M. & Soares, M. B. (1996) *Genomics* **33,** 151–152.
19. Hall, K. T., Boumsell, L., Schultze, J. L., Boussiotis, V. A., Dorfman, D. M., Cardoso, A. A., Bensussan, A., Nadler, L. M. & Freeman, G. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 11780–11785.
20. Xu, Y. & Slayter, H. S. (1994) *J. Histochem. Cytochem.* **42,** 1365–1376.
21. Bork, P. & Beckmann, G. J. (1993) *Mol. Biol.* **231,** 539–545.
22. Miyamoto, M., Naruo, K., Seko, C., Matsumoto, S., Kondo, T. & Kurokawa, T. (1993) *Mol. Cell. Biol.* **13,** 4251–4259.
23. Rubartelli, A., Cozzolino, F., Talio, M. & Sitia, R. (1990) *EMBO J.* **9,** 1503–1510.
24. Tarantini, F., Gamble, S., Jackson, A. & Maciag, T. (1995) *J. Biol. Chem.* **270,** 29039–29042.
25. Mignatti, P., Morimoto, T. & Rifkin, D. B. (1992) *J. Cell. Physiol.* **151,** 81–93.
26. Usuki, K., Heldin, N. E., Miyazono, K., Ishikawa, F., Takaku, F., Westermark, B. & Heldin, C. H. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7427–7431.
27. Rubartelli, A. & Sitia, R. (1997) in *Unusual Secretory Pathways: From Bacteria to Man*, eds. Kuchler, A., Rubartelli, A. & Holland, B. (R. G. Landes Co., Austin, TX), pp. 87–114.
28. Davis, C. G. (1990) *New Biol.* **2,** 410–419.
29. D'Andrea, A. D., Fasman, G. D. & Lodish, H. F. (1990) *Curr. Opin. Cell Biol.* **2,** 648–651.
30. Li, S. W., Sieron, A. L., Fertala, A., Hojima, Y., Arnold, W. V. & Prockop, D. J. (1996) *Proc. Natl. Acad. Sci. USA* **93,** 5127–5130.
31. Weis, W. I. & Drickamer, K. (1996) *Annu. Rev. Biochem.* **65,** 441–473.
32. Stuhler, G. & Schlossman, S. F. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 622–627.
33. Ridge, J. P., Di Rosa, F. & Matzinger, P. (1998) *Nature (London)* **393,** 474–478.
34. Proost, P., De Meester, I., Schols, D., Struyf, S., Lambeir, A. M., Wuyts, A., Opdenakker, G., De Clercq, E., Scharpe, S. & Van Damme, J. (1998) *J. Biol. Chem.* **273,** 7222–7227.