The serpin-enzyme complex (SEC) receptor mediates the neutrophil chemotactic effect of alpha-1 antitrypsin-elastase complexes and amyloid-beta peptide.

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Research Article

The serpin-enzyme complex (SEC) receptor mediates catabolism of alpha 1-antitrypsin (alpha 1-AT)-elastase complexes and increases in synthesis of alpha 1-AT in cell culture. The SEC receptor recognizes a pentapeptide domain on alpha 1-AT-elastase complexes (alpha 1-AT 370-374), and the same domain in several other serpins, amyloid-beta peptide, substance P, and other tachykinins. Thus, it has also been implicated in the biological properties of these ligands, including the neurotoxic effect of amyloid-beta peptide. In this study, we examined the possibility that the SEC receptor mediates the previously described neutrophil chemotactic activity of alpha 1-AT-elastase complexes, and whether the other ligands for the SEC receptor have neutrophil chemotactic activity. The results show that 125I-peptide 105Y (based on alpha 1-AT 359-374) binds specifically and saturably to human neutrophils, and the characteristics of this binding are almost identical to that of monocytes and hepatoma-derived hepatocytes. Peptide 105Y and amyloid-beta peptide mediate chemotaxis for neutrophils with maximal stimulation at 1-10 nM. Mutant or deleted forms of peptide 105Y, which do not bind to the SEC receptor, have no effect. The neutrophil chemotactic effect of alpha 1-AT-elastase complexes is blocked by antiserum to peptide 105Y and by antiserum to the SEC receptor, but not by control antiserum. Preincubation of neutrophils with peptide 105Y or substance P completely blocks the chemotactic activity of amyloid-beta peptide, but not […]

The Serpin-Enzyme Complex (SEC) Receptor Mediates the Neutrophil Chemotactic Effect of α-$_1$ Antitrypsin-Elastase Complexes and Amyloid-β Peptide

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Abstract

The serpin-enzyme complex (SEC) receptor mediates catabolism of α_1-antitrypsin (α_1-AT)-elastase complexes and increases in synthesis of α_1-AT in cell culture. The SEC receptor recognizes a pentapeptide domain on α_1-AT-elastase complexes (α_1-AT 370-374), and the same domain in several other serpins, amyloid-β peptide, substance P, and other tachykinins. Thus, it has also been implicated in the biological properties of these ligands, including the neurotoxic effect of amyloid-β peptide. In this study, we examined the possibility that the SEC receptor mediates the previously described neutrophil chemotactic activity of α_1-AT-elastase complexes, and whether the other ligands for the SEC receptor have neutrophil chemotactic activity. The results show that ^{125}I-peptide 105Y (based on α_1-AT 359-374) binds specifically and saturably to human neutrophils, and the characteristics of this binding are almost identical to that of monocytes and hepatoma-derived hepatocytes. Peptide 105Y and amyloid-β peptide mediate chemotaxis for neutrophils with maximal stimulation at 1–10 nM. Mutant or deleted forms of peptide 105Y, which do not bind to the SEC receptor, have no effect. The neutrophil chemotactic effect of α_1-AT-elastase complexes is blocked by antiserum to peptide 105Y and by antiserum to the SEC receptor, but not by control antiserum. Preincubation of neutrophils with peptide 105Y or substance P completely blocks the chemotactic activity of amyloid-β peptide, but not that of FMLP. These results, therefore, indicate that the SEC receptor can be modulated by homologous desensitization and raise the possibility that pharmacological manipulation of this receptor will modify the local tissue response to inflammation/injury and the neuropathologic reaction of Alzheimer's disease. (*J. Clin. Invest.* 1992. 90:1150–1154.) Key words: serpins • protease inhibitors • inflammation • Alzheimer's disease • Down's syndrome

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Introduction

Neutrophil elastase is capable of degrading most of the constituents of the extracellular matrix and, therefore, is thought to play an important role in tissue remodeling during homeostasis and tissue injury/inflammation (1). α_1-antitrypsin (α_1-AT) is the major physiological inhibitor of neutrophil elastase, forming stable inhibitory complexes with this protease. On the basis of previous observations that show that α_1-AT-elastase complexes are subject to rapid in vivo catabolism by the liver (2, 3), that these complexes stimulate increases in synthesis of α_1-AT in cell culture (4, 5) and are chemotactic for neutrophils (6, 7), the existence of a receptor or receptors for α_1-AT-elastase complexes has been predicted. We have recently identified a cell surface protein, the serpin-enzyme complex (SEC)[1] receptor (8), which validates this prediction. It is a cell surface protein that has a ligand binding subunit of ~ 78 kD and that recognizes α_1-AT only when this inhibitor molecule undergoes structural rearrangement, such as that which accompanies inactivation of elastase. The SEC receptor is capable of mediating endocytosis and catabolism of α_1-AT-elastase complexes in cell culture and binding to the SEC receptor mediates increases in synthesis of α_1-AT (8-11). A pentapeptide domain in the carboxyl terminus of α_1-AT is sufficient for α_1-AT-elastase complexes to bind to the SEC receptor (9). Through a homologous pentapeptide domain, several other serpin-enzyme complexes, the amyloid-β peptide, bombesin, and tachykinins also bind to the SEC receptor (10). In the current study, we examined the possibility that this receptor also mediates the neutrophil chemotactic activity of α_1-AT-elastase complexes, and whether other ligands, such as the amyloid-β peptide, are chemotactic via activation of the SEC receptor.

Methods

Materials. Peptides 105Y, 105C, and 105C-C, and amyloid-β peptide 25–35 were synthesized by the solid phase method, purified, and subjected to amino acid composition and sequence analysis, as previously described (8). Purified human α_1-AT and human sputum elastase were purchased from Athens Research and Technology (Athens, GA) and Elastin Products (Pacific, MO), respectively. Substance P was purchased from Sigma Chemical Co. (St. Louis, MO).

Isolation of neutrophils. Neutrophils were purified from normal donor blood by sedimentation on 6% Dextran (Pharmacia Fine Chemi-

1. *Abbreviation used in this paper:* SEC, serpin-enzyme complex.

cals, Piscataway, NJ) in PBS. The upper layer was collected and centrifuged over Ficoll (Pharmacia Fine Chemicals) for 20 min at 2,000 rpm. The pellet was washed two times in PBS/3 mM EDTA and subjected to hypotonic conditions to clear the neutrophils of contaminating red cells. The remaining cells were > 95% viable by trypan blue exclusion.

Determination of cell surface receptor binding. Neutrophils were resuspended in PBS supplemented with 1 mg/ml BSA, 3 mM EDTA, 0.05% Tween 80, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml pepstatin. 0.5 ml samples of 2×10^6 neutrophils were incubated for 1 h at room temperature with various concentrations of chloramine T labeled ^{125}I-peptide 105Y in the presence or absence of 100-fold molar excess unlabeled peptide 105Y. After incubation, bound labeled peptide was separated from free labeled peptide by rapid centrifugation. Cell pellets were washed in incubation buffer. Bound ^{125}I peptide 105Y was counted by gamma scintillation. Specific binding was defined as the difference between total binding in the absence of cold peptide 105Y and nonspecific binding in the presence of cold peptide 105Y.

Chemotaxis. Chemotaxis was determined in modified Boyden chambers, as previously described (12). Briefly, 1.2×10^6 neutrophils/ml were placed in the upper compartment and separated from chemoattractants in the lower compartment by a 2-μm pore size filter (Nucleopore Corp., Pleasanton, CA) overlaying a 0.45 μm pore size filter (Millipore Corp., Bedford, MA). After 1 h the chambers were disassembled and the membranes were stained with hematoxylin. Chemotaxis was quantified by counting at high dry magnification (×400) the number of cells that migrated to the interface between the membranes (expressed as cells per high power grid). Five fields were counted for each experiment. The number of cells migrating in the control was considered background and subtracted for the final reported results. Experiments were performed in triplicate.

For experiments in which the neutrophil chemotactic effects of α_1-AT-elastase complexes are blocked by antisera, the α_1-AT-elastase complexes were prepared according to the previously described protocol (8). Antiserum to keyhole-limpet hemocyanin-coupled peptide 105Y was prepared by hyperimmunizing New Zealand White rabbits. Specific antibody titers were determined by ELISA. Both anti-peptide 105Y and anti-SEC receptor antiserum (Perlmutter, D. H., G. Joslin, and R. J. Fallon, manuscript in preparation) were shown to specifically block binding of ^{125}I peptide 105Y and ^{125}I elastase-α_1-AT complexes to HepG2 cells.

For desensitization experiments, neutrophils were preincubated for 15 min at 37°C with medium alone, medium supplemented with peptide 105Y (10^{-8} M), or medium supplemented with substance P (10^{-8} M). The cells were then washed three times with assay medium and assayed for a chemotactic response to amyloid-β peptide (25-35) in several different concentrations (10^{-11}–10^{-7} M) or to f-metleuphe, FMLP, in one concentration (10^{-8} M).

Results

First, we examined the possibility that the SEC receptor was expressed on human neutrophils (Figs. 1 *B* and 1 *C*). Human neutrophils were purified from normal donor blood. The expression of the SEC receptor on neutrophils was assessed by radioligand binding assays (Fig. 1 *B*) using a synthetic ^{125}I-labeled peptide 105Y (SIPPEVKFNKPFVYLI) derived from α_1-AT sequence 359-374. A saturation binding curve from one donor is shown in Fig. 1 *B*. There is specific and saturable binding with the point of half-maximal saturation at ~ 40–50 nM, almost identical to that in HepG2 cells (8). A saturation binding curve from another donor is shown in Fig. 1 *C*. This curve was subjected to Scatchard analysis (*inset*), and predicted a K_d of 43 nM with ~ 13,000 plasma membrane receptors per cell. Saturation binding curves in three other donors have had almost identical characteristics, although receptor

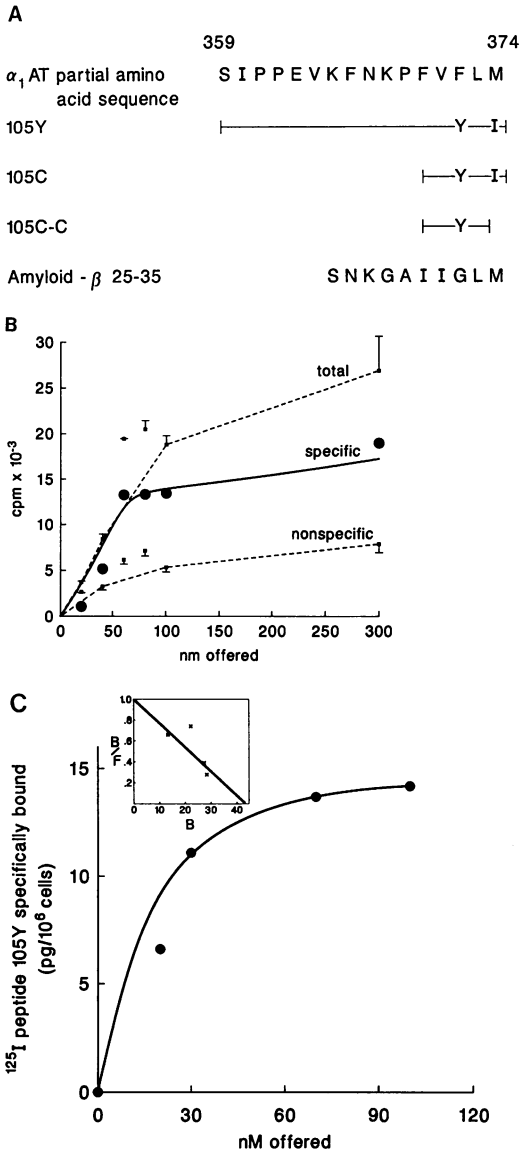
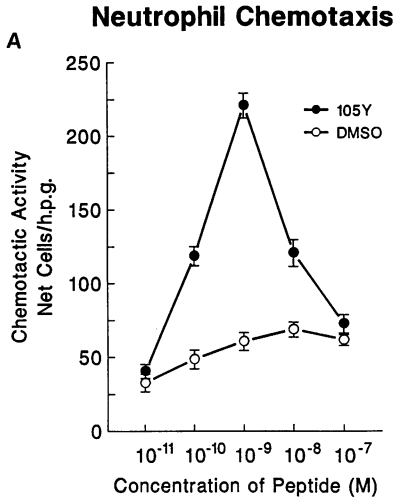


Figure 1. Peptide 105Y binds to the SEC receptor on human neutrophils. (*A*) Amino acid sequences of α_1-AT and several synthetic peptides. (*B*) Saturation binding curve for ^{125}I peptide 105Y binding to human neutrophils from one normal donor. (*C*) Saturation binding curve of specifically bound ^{125}I peptide 105Y to human neutrophils of another donor with Scatchard plot (*inset*) predicts a K_d of 43 nM and 13,000 receptor copies per cell.

number has been ~ 13,000 in one and 400,000–500,000 in two of these.

Next, we examined the possibility that peptide 105Y is chemotactic for neutrophils (Fig. 2 *A*). Neutrophils were placed in the upper compartment of modified Boyden chambers separated from peptide 105Y, FMLP, or solvent medium by a 2-μm pore filter overlaying a 0.45-μm pore filter. Peptide 105Y elicited chemotaxis with maximal stimulation at a concentration of 10^{-9} M. The solvent in these experiments (DMSO) did not stimulate migration of neutrophils. The magnitude of the effect was comparable in potency to that of 10^{-8} M FMLP. The specificity of this effect was further examined with the shorter synthetic peptides 105C and 105C-C (Fig. 2 *B*). Pentapeptide



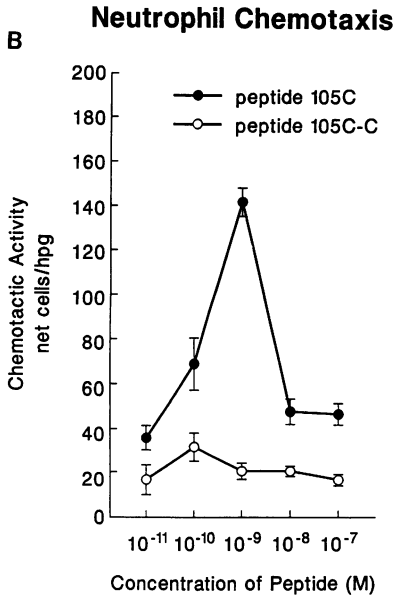


Figure 2. Stimulation of neutrophil chemotaxis by synthetic peptides. (*A*) Peptide 105Y (*closed circles*), carrier solvent (*open circles*). (*B*) Peptide 105C (*closed circles*), and peptide 105C-C (*open circles*).

105C (FVYLI) corresponds to the minimal SEC receptor-binding domain of α_1-AT-elastase complexes (4). Peptide 105C-C (FVYL) lacks the carboxyl-terminal residue of peptide 105C and does not bind to the SEC receptor. Peptide 105C stimulated migration of neutrophils with a maximal effect at 10^{-9} M peptide. Peptide 105C-C had no effect (Fig. 2 *B*). The specificity of the chemotactic activity was, therefore, consistent with the specificity of the SEC receptor.

To further demonstrate that the chemotactic property of α_1-AT-elastase complexes was mediated by the SEC receptor, we examined the possibility that the property could be blocked by specific antiserum to peptide 105Y or antiserum to the SEC receptor (Fig. 3). First, neutrophils were placed in the upper compartments of a modified Boyden chamber separated from α_1-AT-elastase complexes in the lower compartment. α_1-AT-elastase complexes elicited directed migration with maximal stimulation at a concentration of 10^{-8} M α_1-AT initially added to the reaction mixture. This is a somewhat higher effective concentration than that required with synthetic peptides (Fig. 2). However, it is not possible to determine whether this difference is real or apparent because the actual concentration of ligand presented to the binding site by the α_1-AT-elastase complex reaction mixture cannot yet be measured. The magnitude of the chemotactic effect of α_1-AT-elastase complexes was comparable in potency to that of FMLP. The chemotactic effect of α_1-AT-elastase complexes, but not that of FMLP, was completely blocked by coadministration of anti-peptide 105Y in the lower compartment (Fig. 3). Moreover, the effect of α_1-AT-elastase complexes was completely blocked when neutrophils were preincubated with antiserum to the SEC receptor, but not when preincubated with a control antiserum.

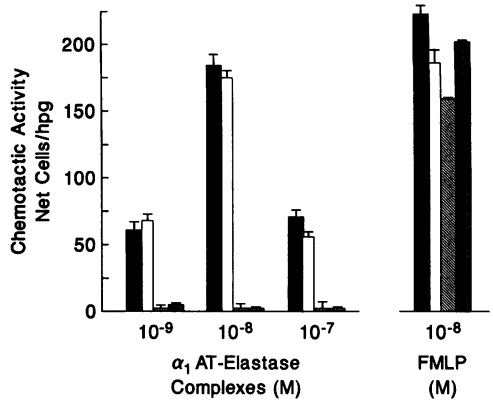


Figure 3. Effect of specific antibodies on neutrophil chemotactic effect of α_1-AT-elastase complexes. Chemotaxis assays were performed as described above with α_1-AT-elastase complexes in three different concentrations (10^{-9}–10^{-7} M) or f-Met-Leu-Phe (FLMP) in one concentration (10^{-8} M) as the putative chemotactic factors. In each case, the putative chemotactic factor was put in the lower chamber in medium alone (*solid bars*) or in medium supplemented with rabbit antiserum to peptide 105Y in a 1:50 dilution (*hatched bars*). In separate samples the putative chemotactic factor was put in the lower chamber in medium alone and the neutrophils added to the upper chamber in medium containing a control antiserum (rabbit antiserum to guinea pig C3 [*open bars*]) or the neutrophils added to the upper chamber in medium containing anti-SEC receptor antiserum in a 1:50 dilution (*dotted bars*). ■ Medium alone; □ medium + control antiserum; ▨ medium + anti-peptide antiserum; □ medium + anti-receptor antiserum.

We next examined the possibility that other ligands for the SEC receptor, such as amyloid-β peptide, had neutrophil chemotactic properties and whether these properties could be desensitized (Fig. 4). In a previous study, we have shown that amyloid-β peptide 1-42 binds to the SEC receptor (10). In our subsequent studies, we have shown that amyloid-β peptide 25-35 and 30-35 bind to the SEC receptor as well as amyloid-β peptide 1-42 (Joslin, G., R. J. Fallon, and D. H. Perlmutter, unpublished results), thereby localizing the receptor binding domain to the pentapeptide region which is most homologous to substance P, peptide 105Y, and α_1-AT sequences. Here, we used amyloid-β peptide 25-35 and found it to elicit robust neutrophil chemotaxis, maximal at 10^{-9}–10^{-8} M, and completely abrogated by pretreatment with peptide 105Y (Fig. 4 *A*). Moreover, the neutrophil chemotactic effect of amyloid-β peptide was completely abrogated by preincubation of the neutrophils with substance P (Fig. 4 *B*). Preincubation of neutrophils with peptide 105Y or substance P did not block the chemotactic effect of FMLP. Accordingly, peptide 105Y, amyloid-β peptide, and substance P bind to the same receptor on neutrophils, elicit potent chemotactic responses at low receptor occupancy, and elicit homologous desensitization of the SEC receptor.

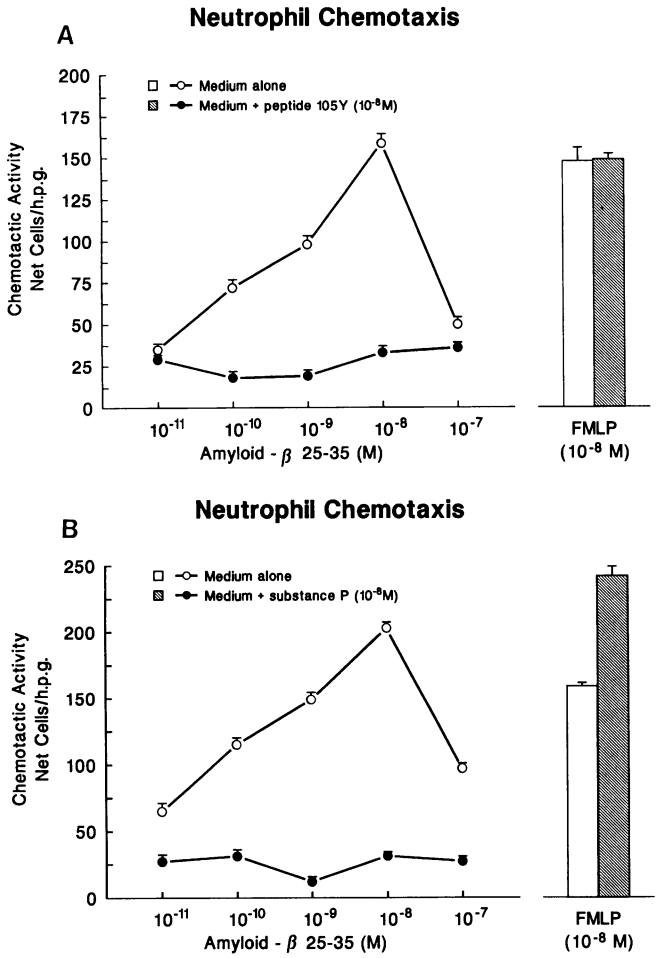


Figure 4. Neutrophils are desensitized to the chemotactic effect of amyloid-β peptide by incubation with other ligands for the SEC receptor. (*A*) Neutrophils were preincubated for 15 min at 37°C with either medium alone (*open circles, open bars*) or medium supplemented with peptide 105Y in a concentration of 10^{-8} M (*closed circles, hatched bars*). (*B*) Neutrophils were preincubated with medium alone (*open circles, open bars*) or medium supplemented with substance P in a concentration of 10^{-8} M (*closed circles, hatched bars*). Neutrophils were then centrifuged and washed three times and then added to the upper chamber across from amyloid-β peptide (25–35) in several different concentrations (10^{-11}–10^{-7} M) or across from FMLP in one concentration (10^{-8} M).

Discussion

These studies provide further evidence for the important role of the SEC receptor in the host response to tissue injury/inflammation. This receptor mediates increases in de novo synthesis of α_1-AT in response to α_1-AT-elastase complexes (8), probably mediates in vivo clearance/catabolism of α_1-AT-elastase complexes (11), and herein is shown to mediate directed migration of neutrophils toward α_1-AT-elastase complexes. The SEC receptor also recognizes α_1-AT after it has undergone limited proteolysis by other important constituents of the inflammatory reaction including metallo-elastases (13). Moreover, the SEC receptor recognizes several other serpin-enzyme complexes that are likely to be present at sites of inflammation (8). A recent study investigating the chemotactic properties of $\alpha_1$antichymotrypsin-cathepsin G complexes indicates that complexes, but not native $\alpha_1$antichymotrypsin, are chemotactic for neutrophils (14). We have previously shown that $\alpha_1$antichymotrypsin-cathepsin G complexes, but not native $\alpha_1$antichymotrypsin, bind to the SEC receptor (8), making it likely that the activity observed for $\alpha_1$antichymotrypsin-cathepsin G complexes is mediated by the SEC receptor. Heparin cofactor II, another serpin having a region in its carboxyl terminus that is similar to the SEC receptor-binding domain, is chemotactic for neutrophils, but a structurally distinct region in the amino terminal domain has been implicated in its chemotactic properties (15). Further studies will be necessary to determine whether two separate regions of heparin cofactor II can mediate neutrophil chemotaxis.

Amyloid-β peptide is a major proteinaceous constituent of the extracellular deposits found in Alzheimer's disease and Down's syndrome (16). It is not yet entirely clear how the amyloid-β peptide is translocated from the intramembranous domain of the transmembrane amyloid precursor protein to the extracellular space. Most of the evidence suggests that the amyloid-β peptide is generated by abnormal proteolytic processing of the amyloid precursor protein, which leads to extracellular deposition and represents the primary pathophysiologic element of these conditions. Several recent studies have demonstrated a neurotrophic/neurotoxic effect for amyloid-β peptide (17) or cores of amyloid plaques (18) by administration to cultured neurons or by injection into the brains of experimental animals. In one series of studies, these effects have been attributed to amyloid-β peptide 25-35 region and have been blocked by substance P. Thus, in a recent review of Alzheimer's disease, the SEC receptor is implicated in mediating the neurotoxic effect of amyloid-β peptide (19).

Although it has not been emphasized in the description of Alzheimer's disease, several studies have reported an inflammatory response surrounding amyloid plaques. In studies of immune associated antigens in human postmortem samples, several reports show expression of HLA-DR major histocompatibility antigen on microglial cells adjacent to amyloid plaques (20). Moreover, microglial cell proliferation and scavenging activity, as well as T cell infiltration, was reported at plaque sites (21). Because the SEC receptor has been demonstrated on the surfaces of cells of myeloid lineage, including monocytes and neutrophils, it is possible that SEC receptor expression on microglial cells plays a role in this local inflammatory response. Taken together with the previous observations about Alzheimer's disease, the present report suggests the working hypothesis that the SEC receptor plays a role in the pathophysiology of this disease by mediating clearance and intracellular proteolysis of the amyloid-β peptide, a neurotrophic/neurotoxic effect of the amyloid-β peptide, and/or a local inflammatory response to the amyloid-β peptide.

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