

# Molecular Characterization of an Autoallergen, Hom s 1, Identified by Serum IgE from Atopic Dermatitis Patients<sup>1</sup>

Rudolf Valenta,\* Susanne Natter,<sup>2\*</sup> Susanne Seiberler,<sup>2\*</sup> Sibylle Wichlas,† Dieter Maurer,† Michael Hess,§ Margit Pavelka,§ Monika Grote,†† Fatima Ferreira,\*\* Zsolt Szepefalusi,\* Peter Valent,‡ and Georg Stingl†

\*Institute of General and Experimental Pathology, †Department of Dermatology, Division of Allergy, Immunology and Infectious Diseases, ‡Department of Internal Medicine I, Division of Hematology and Hemostasiology, Vienna General Hospital, University of Vienna Medical School, Vienna, Austria; §Institute of Histology and Embryology, University of Innsbruck Medical School, Innsbruck, Austria; ¶Institute of Biotechnology, University of Helsinki, Helsinki, Finland; \*\*Institute of Developmental Biology, University of Salzburg, Salzburg, Austria; ††Institute of Medical Physics, University of Munster Medical School, Munster, Germany

Atopy is a genetically determined disorder that affects 10%–20% of the population. Many symptoms of patients with atopy (allergic rhinitis, conjunctivitis, asthma, and anaphylaxis) result from events occurring after cross-linking of cell-bound IgE by *per se* innocuous environmental antigens. The frequently raised hypothesis that autosensitization can also be a pathogenetic factor in atopy, gained support by our recent demonstration of IgE antibodies against human proteins in atopic dermatitis patients. To unravel the molecular nature of IgE-defined autoantigens, we used serum IgE from atopic dermatitis patients to screen a human epithelial cDNA expression library. One of the cDNA-encoding IgE-reactive products contained 1501 bp of a 2274 bp open-reading frame finally identified by sequence analysis of two additional cDNA clones resulting from oligonucleotide screening. The IgE-defined autoantigen, designated Hom s 1,

exhibited an almost complete sequence identity with recently described antigen recognized by cytotoxic cells of a squamous cell carcinoma patient. Purified recombinant Hom s 1 specifically bound IgE from patients with severe atopy. When used as immunogen in rabbit recombinant Hom s 1 gave rise to an anti-serum that reacted with a cytoplasmic protein exhibiting a broad cellular and tissue reactivity (skin, lung >> gastrointestinal tract >> muscle, brain) and identified a 55 kDa protein blotted serum IgE preparations. The attractive possibility remains that the Hom s 1-triggered IgE response contributes to the events resulting in allergic tissue inflammation. If so, the respective recombinant molecule may serve as a paradigmatic tool for the diagnosis and treatment of patients with "intrinsic" atopy. **Key words:** atopy, cDNA cloning/IgE autoimmunity/recombinant autoallergen. *J Invest Dermatol* 111:1178–1183, 1998

**A**topy, a major health problem in industrialized countries, describes the genetically determined tendency of a given individual to develop hypersensitivity reactions to essentially innocuous environmental substances, e.g., pollen, mite, animal hair/dander, and mould proteins (Casolaro *et al*, 1996). Certain clinical manifestations of atopy such as allergic rhinitis, conjunctivitis and asthma are thought to result from IgE-mediated, immediate reactions, i.e., allergen-induced cross-linking of mast cell/basophil-bound IgE and subsequent release of their mediators. The major skin affection of this disorder, i.e., atopic dermatitis (Leung *et al*, 1993), resembles other eczematous diseases and exhibits several features of a T cell-mediated, delayed-type hypersensitivity reaction. Recent evidence indicates that this response is far greater in magnitude when the allergen is taken up by the antigen-presenting cell in the

context of IgE rather than in its unbound form (Mudde *et al*, 1995; Maurer *et al*, 1995, 1996).

Although the occurrence of the clinical reaction can usually be correlated with the exposure to the respective substance, certain patients exhibit signs of atopic disease even in the absence of environmental allergens. Early clinical studies describing immediate type skin reactions to human proteins (Hampton and Cooke, 1941), together with the more recent findings of structural and immunologic similarities between environmental allergens and human proteins (Valenta *et al*, 1993; Cramer *et al*, 1996; Fedorov *et al*, 1997), indicated that IgE auto-reactivity could be of pathogenetic importance in atopy.

In a recent study we searched, by western blotting, for the presence of autoreactive IgE antibodies in sera from atopic individuals, patients with other immunologic disorders, and healthy control persons. We found that mainly sera from atopic dermatitis (AD) patients displayed IgE reactivity against a wide variety of antigens expressed in histogenetically different cell types (Valenta *et al*, 1996).

To reveal the molecular nature of IgE-defined autoantigens, we used serum IgE from two AD patients with pronounced IgE reactivity to western-blotted human proteins for the screening of a cDNA expression library constructed from the human epithelial cell line A43 in phage  $\lambda$ gt11. Several of the cDNA isolated via IgE coded for known intracellular proteins. These IgE-defined autoantigens included: (i) the C-terminal portion of the  $\alpha$ -chain of the human nascent polypeptide associated complex, a protein required for signal sequence-specific

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Reprint requests to: Dr. Rudolf Valenta, Institute of General and Experimental Pathology, Vienna General Hospital, University of Vienna Medical School, Waehringer Guertel 18-20, A-1090 Vienna, Austria.

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<sup>2</sup>Susanne Natter and Susanne Seiberler contributed equally to the work and are listed in alphabetical order.

sorting and translocation of intracellular proteins (Wiedmann *et al.*, 1994); (ii) BCL7, a putative oncogene; (iii) the central helical portion of human cytokeratin type II (Tyner *et al.*, 1985); and (iv) a calcium-binding protein (Natter *et al.*, 1998). These proteins represent biologically important intracellular moieties that were not yet described as autoantigens (Tan, 1991; Nousari and Anhalt, 1995). The cDNA encoding the most frequently recognized IgE autoantigen, designated *Hom s 1*, displayed an almost complete sequence identity with SART-1, a recently described antigen recognized by cytotoxic T cells of a patient suffering from squamous esophageal cancer (Nakao *et al.*, 1995; Shichijo *et al.*, 1998). In this study, the isolation, molecular characterization, expression in *Escherichia coli*, and purification of *Hom s 1* are reported. The tissue expression and subcellular localization of *Hom s 1* were studied and the capacity of purified recombinant *Hom s 1* to bind IgE antibodies from AD patients was investigated.

## MATERIALS AND METHODS

**Phage libraries, *E. coli* strains, plasmids** cDNA libraries constructed from the human cell line A431 and human testis in phage  $\lambda$ gt11 were purchased from Clontech (Palo Alto, CA). *Escherichia coli* strains Y1090 [*hsd* ( $r_k^- m_k^-$ ) *lac* U169, *proA*+, *lon*-, *araD* 139, *StrA*, *Sup F* *trpC22*:Tn10(pMC9)] and Y1089 [*hsd* ( $r_k^- m_k^-$ ) *lac* U169, *proA*+, *lon*-, *araD* 139, *StrA*, *hflA* 150 chr.Tn10 (pMC9)] were obtained from Amersham (Bucks, U.K.) and *E. coli* XL-1Blue *recA1 endA1 gyrA96 thi-1 hsdR17 supE44 mlA1 lac* [F' *proAB lacZ*:EM15 Tn10 (Tet)<sup>r</sup>] were from Stratagene (La Jolla, CA). Plasmid pUC18 was from Boehringer (Mannheim, Germany).

**Cell lines, tissue specimens, anti-sera** The human epithelial cell line A431, established from an epidermoid mammary carcinoma, was obtained from the American Type Culture Collection (Rockville, MD). The human mast cell line HMC-1 originated from a patient suffering from mast cell leukemia (Butterfield *et al.*, 1988). Cells were grown in RPMI 1640 medium supplemented with 2 mM L-glutamine, 50 mM  $\beta$ -ME, 100 U penicillin per ml, 100  $\mu$ g streptomycin (Gibco, Gaithersburg, MD) per ml, and 10% fetal calf serum (Sera Lab, Crawley Down, U.K.). Human tissue samples were obtained from biopsies taken either *ex vivo* (bone marrow, bone, skin, uterus, lung, colon, stomach, skeletal muscle, heart muscle) or immediately *post mortem* (liver, brain) for routine histology. Rabbit anti-sera directed against human actin and human histone H2B were purchased from Pierce (Rockford, IL) and from Chemicon (Temecula, CA), respectively. The rabbit anti-birch profilin anti-serum RP3 has been described previously (Valenta *et al.*, 1993). *Hom s 1*-specific antibodies were generated by immunization of a rabbit with the *Hom s 1*- $\beta$ -gal fusion protein and protein G affinity purification of serum Ig.

**Protein extracts, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunoblotting** Protein extracts were prepared by homogenizing cells/tissue specimens in SDS-sample buffer (Laemmli, 1970). Protein extracts were boiled for 5 min, separated by 12.5% SDS-PAGE, and stained with Coomassie Blue (Bradford, 1976). Proteins were transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) by electroblotting (Towbin *et al.*, 1979), and membrane-bound proteins were exposed to human IgE or rabbit anti-sera as previously described (Valenta *et al.*, 1992).

**Isolation and molecular characterization of *Hom s 1*-encoding cDNA clones** *Hom s 1* cDNA (clone a) was isolated by IgE immunoscreening of a A431 cDNA expression library as described (Valenta *et al.*, 1991). cDNA clones (b and c) were obtained by screening a cDNA library from human testis with the <sup>32</sup>P-labeled oligonucleotide 5' GAG GCG GGC ACC AAG GAG GAG CCC GTG ACA GCT G 3' synthesized according to the DNA sequence of clone a (Sambrook *et al.*, 1989). cDNA of clones a, b, and c were excised from the phage DNA using Eco R I, subcloned into the plasmid pUC18, and both DNA strands were sequenced using forward and reversed primers (Boehringer), internal primers (Pharmacia-Biotech, Uppsala, Sweden), and <sup>35</sup>S-dCTP (NEN, Stevenage, U.K.) (Sanger *et al.*, 1977). The correct orientation of clone a was confirmed by sequencing a Sac I fragment obtained from the phage DNA with  $\lambda$ gt11 sequencing primers. The cDNA sequence of clone c was confirmed by automated DNA sequencing (Schmidheim, Windisch, Switzerland). The amino acid sequence of *Hom s 1* was established using the McVector program (Kodak, Rochester, NY). Molecular weight and isoelectric point of the *Hom s 1* protein as well as its secondary structure and sequence motifs were also analyzed using the McVector program. The cDNA and deduced amino acid sequence of *Hom s 1* were compared with the sequences deposited in GENBANK using the BLAST program. A431 genomic DNA and HMC-1 RNA were isolated by cesium chloride density centrifugation (Sambrook *et al.*, 1989). Twenty micro-

gram aliquots of genomic DNA were digested with either Sac I, Hind III, or Bam H I, separated by 1% agarose gel electrophoresis, and blotted onto nitrocellulose. For control purposes, 5  $\mu$ g of Pst I-digested lambda phage DNA was used. Approximately 20  $\mu$ g of HMC-1 RNA were separated by denaturing 1.5% agarose gel electrophoresis and blotted onto nitrocellulose. Blots were hybridized with the <sup>32</sup>P-dCTP-labeled cDNA of clone a (Fenberg and Vogelstein, 1983).

**Expression, purification, and analysis of the IgE reactivity of recombinant *Hom s 1*** Recombinant *Hom s 1* was expressed as a  $\beta$ -gal fusion protein in *E. coli* Y1089 (Huynh *et al.*, 1985). The fusion protein as well as  $\beta$ -gal alone were affinity purified using sepharose-coupled anti- $\beta$ -gal antibodies (Promega, Madison, WI). Approximately 5  $\mu$ g per cm gel of the purified proteins were separated by 12.5% SDS-PAGE (Laemmli, 1970) and blotted onto nitrocellulose (Towbin *et al.*, 1979). Nitrocellulose-bound *Hom s 1*- $\beta$ -gal fusion protein or  $\beta$ -gal alone was incubated with sera from AD patients, serum from a nonallergic individual, buffer without serum, or a supernatant from the human IgE myeloma cell line U266 (Nilsson *et al.*, 1970). Bound IgE was detected with <sup>125</sup>I-labeled anti-IgE antibodies (RAST, Pharmacia) as described (Valenta *et al.*, 1992). IgE epitopes shared by natural and recombinant *Hom s 1* were studied by IgE absorption experiments. A 1:10 diluted *Hom s 1*-reactive serum was preabsorbed with 5  $\mu$ g purified *Hom s 1*- $\beta$ -gal or  $\beta$ -gal per ml prior to exposure to nitrocellulose-blotted A431 extracts.

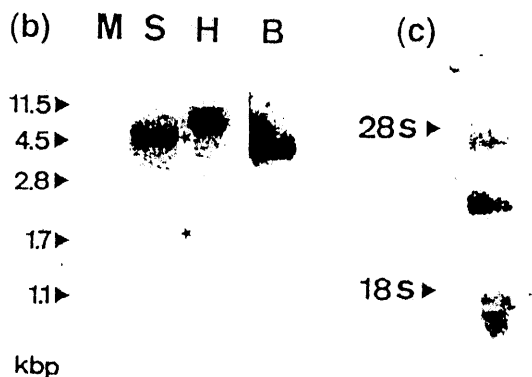
**Immunohistochemistry** Punch biopsies were obtained from lesional skin of AD patients and from normal skin of healthy individuals. Five millimeter acetone-fixed cryostat sections were exposed to equal concentrations of rabbit anti-*Hom s 1* Ig or preimmune Ig. Bound antibodies were detected with biotinylated donkey anti-rabbit F(ab')<sub>2</sub> (Amersham) followed by avidin/biotinylated horseradish peroxidase complexes (Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany), visualized with 3-amino-9-ethylcarbazole (Sigma-Aldrich), and sections counterstained with hematoxylin (Merck, Darmstadt, Germany).

To study the ultrastructural localization of *Hom s 1*, HepG2 cells were processed for post-embedding immunoelectron microscopy using cryofixation, cryosubstitution with acetone, and ambient temperature resin embedding (Hess *et al.*, 1995). Eighty nanometer ultrathin sections were labeled with rabbit anti-*Hom s 1* Ig. Bound antibodies were detected with goat anti-rabbit IgG coupled to 10 nm colloidal gold (British Biocell, Cardiff, U.K.).

**Purification and analysis of IgE-anti-*Hom s 1* immune complexes** IgE was affinity purified from sera of two individuals with (DM, SJ) and two without (VR, ZH) western blot-detectable *Hom s 1*-specific IgE antibodies using CNBr-sepharose-coupled monoclonal anti-human IgE antibody LE27 (de Weck and Stadler, 1993). Comparable amounts (20  $\mu$ g per cm gel) of purified anti-IgE precipitates were separated by SDS-PAGE and blotted onto nitrocellulose. Nitrocellulose strips were probed with rabbit anti-*Hom s 1* and, for control purposes, with rabbit preimmune Ig, rabbit Ig to birch profilin (RP3) (Valenta *et al.*, 1993), and <sup>125</sup>I-labeled anti-human IgE antibodies. Bound rabbit antibodies were detected with a <sup>125</sup>I-labeled donkey anti-rabbit anti-serum (Amersham) and visualized by autoradiography.

## RESULTS

**Isolation and characterization of cDNA clones coding for IgE-defined autoantigens** On the basis of the sequence information obtained from the A431-derived *Hom s 1* cDNA (Fig 1a, clone a, 1501 bp), we isolated two additional clones (Fig 1a, clones b, 2305 bp, and c, 2310 bp) by oligonucleotide screening of a human testis cDNA library. The three clones (a, b, c) were identical in their overlapping regions and resulted in a cDNA sequence of 2424 bp length with an internal Sac I restriction site at bp 1313 and a canonical polyadenylation signal 17 bp upstream of the poly A tail (Fig 1a). Within an open reading frame of 2274 nucleotides, we identified, on the basis of the first ATG in the *Hom s 1* sequence, a cDNA coding for a polypeptide of 73.4 kDa deduced molecular weight and a calculated pI of 4.95. Further characteristics of *Hom s 1* included a remarkable abundance of glutamic acid residues (13.9%), a leucine zipper motif, two amidation and two tyrosine kinase interaction sites, two N-glycosylation positions, one glycosaminoglycan-binding domain, as well as a high content of  $\alpha$ -helices as predicted by secondary structure analysis (Fig 1a). Sequence comparison of the *Hom s 1* cDNA with sequences submitted to the data bases revealed an almost complete sequence identity of the *Hom s 1* cDNA with the recently described SART-1 gene encoding a protein recognized by cytotoxic T cells of a squamous cell carcinoma patient. The *Hom s 1* cDNA differs from

[illegible]

The  $^{32}\text{P}$ -labeled *Hom s 1* cDNA hybridized with single bands in Bam HI- and Hind III-digested and two bands in Sac I-digested human

genomic DNA (Fig 1b). This, together with the fact that the three independently obtained cDNA clones contained identical sequences strongly suggests that *Hom s 1* is encoded by a single gene. Hybridization of the  $^{32}\text{P}$ -labeled *Hom s 1* cDNA with nitrocellulose-blotted RNA from the human mast cell line HMC-1, resulted in a band corresponding to a transcript of  $\approx 2500$  nucleotides (Fig 1c). Hybridization of RNA from:

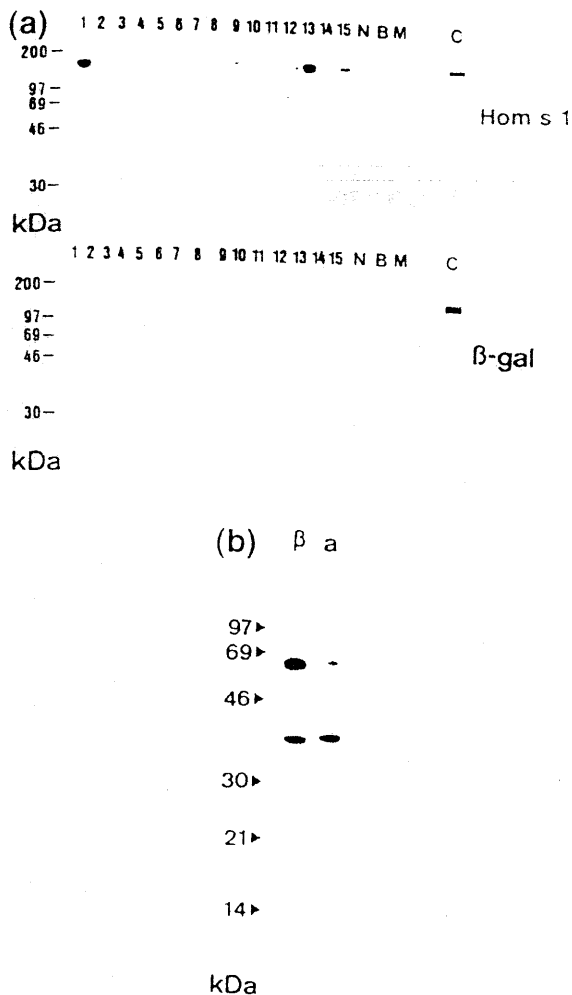


**Figure 2.** IgE binding capacity of recombinant *Hom s 1*. (a) Serum IgE from AD patients recognizes recombinant *Hom s 1*. Recombinant *Hom s 1* was expressed as a  $\beta$ -gal fusion protein in *E. coli* Y1089. Approximately 5  $\mu$ g per cm gel of the purified recombinant proteins were separated by 12.5% SDS-PAGE and blotted onto nitrocellulose. Lane C shows sections of Coomassie-Blue-stained gels containing the purified proteins. Nitrocellulose-blotted *Hom s 1*- $\beta$ -gal fusion protein or  $\beta$ -gal alone was incubated with sera from AD patients (lanes 1–15), serum from a nonallergic individual (lane N), buffer without serum (lane B), or a supernatant from the human IgE-myeloma cell line U266 (Nilsson *et al.*, 1970) (lane M). Bound IgE was detected with  $^{125}$ I-labeled anti-IgE antibodies. The total IgE levels (kU per liter) (CAP, Pharmacia) of patients' sera and of the U266 supernatant were as follows: lane 1, 2050; 2, 1965; 3, 15,350; 4, 12,560; 5, 12,980; 6, 1416; 7, 14; 8, 35; 9, 6180; 10, 133; 11, 19; 12, 12,500; 13, 1756; 14, 1466; 15, 2100; N, 7; M, 1300. (b) Recombinant *Hom s 1* specifically inhibits the binding of *Hom s 1*-specific serum IgE to an A431-derived 55 kDa protein. A 1:10 diluted serum was preabsorbed with 5  $\mu$ g purified *Hom s 1*- $\beta$ -gal(a) or  $\beta$ -gal( $\beta$ ) per ml prior to exposure to nitrocellulose-blotted A431 extracts, and bound IgE was detected.

variety of human cell lines and tissues with the SART-1 cDNA identified transcripts of similar size (Shichijo *et al.*, 1998).

**Expression and purification of recombinant *Hom s 1***  $\lambda$ gt 11 phage containing the clone a cDNA (Fig 1a) were used to infect *E. coli* Y1089, and recombinant *Hom s 1*- $\beta$ -galactosidase ( $\beta$ -gal) fusion protein was affinity purified by sepharose-coupled anti- $\beta$ -gal antibodies. For control purposes,  $\beta$ -gal alone was purified from *E. coli* Y1089 infected with wild-type  $\lambda$ gt 11 phage. As exemplified in Fig 2(a), certain AD sera (six of 65) exhibited specific IgE reactivity of varying intensity with nitrocellulose-blotted recombinant *Hom s 1*. When tested on blotted A431 extracts, an IgE anti-recombinant *Hom s 1*-containing AD serum detected two prominent proteins of 55 and 38 kDa (Fig 2b). Preincubation of this serum with recombinant *Hom s 1*- $\beta$ -gal, but not with  $\beta$ -gal alone, led to a substantial inhibition of the IgE binding to the 55 kDa, but not to the 38 kDa molecule

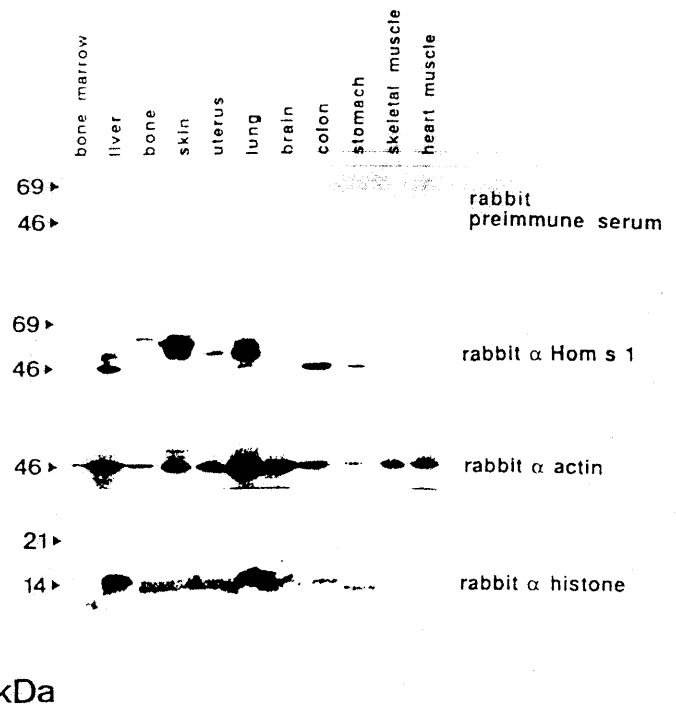


**Figure 3.** Expression of *Hom s 1* in different human tissues. Nitrocellulose-blotted human tissue extracts (bone marrow, liver, bone, skin, uterus, lung, brain, colon, stomach, skeletal, and heart muscle) were exposed to preimmune rabbit Ig as well as to rabbit anti-sera against either *Hom s 1*, actin, or histone H2B. Molecular weights (kDa) are displayed on the left side.

(Fig 2b). These data indicate that the IgE-reactive 55 kDa protein is indeed *Hom s 1* and demonstrate that the recombinant autoantigen shares many of the relevant IgE epitopes with its natural counterpart.

**Tissue expression and subcellular localization of *Hom s 1*** To determine the *in vivo* expression of *Hom s 1*, we raised a rabbit anti-serum against purified recombinant *Hom s 1* to probe nitrocellulose-blotted protein extracts from various human tissues. By western blot, this anti-serum reacted specifically with *Hom s 1* expressed as a  $\beta$ -gal or glutathione S-transferase fusion protein (data not shown). Prominent anti-*Hom s 1*-reactive bands were observed in skin, liver, lung, and colon extracts. No (brain) or only weak (bone marrow, bone, uterus, stomach, skeletal muscle, heart muscle) anti-*Hom s 1* immunoreactivity was noted in the other tissue extracts tested (Fig 3). Rabbit anti-*Hom s 1*-reactive bands of considerable molecular weight variation were observed in the individual tissue specimens investigated. They included 46 kDa polypeptides in the gastrointestinal tract, 55–60 kDa moieties in skin, bone, uterus, and lung, and 80–85 kDa muscle proteins. In contrast, preimmune Ig displayed no reactivity within this molecular weight range (Fig 3). The semiquantitative comparison of the anti-*Hom s 1* immunoreactivity with that of anti-sera directed against other ubiquitously expressed antigens (i.e., actin, histone) indicated that skin is the richest source of *Hom s 1* (Fig 3).

We therefore analyzed by immunohistochemistry the nature and distribution of *Hom s 1*-expressing cells within this organ. Anti-*Hom s 1* immunostaining of tissue sections from lesional skin biopsies of AD patients (Fig 4A), as well as from normal skin of healthy persons (Fig 4C), showed similar reaction patterns. Within the epidermis, the cytoplasm of suprabasal rather than basal keratinocytes was prominently labeled. Within the dermis, *Hom s 1*-expressing cells included fibroblasts and endothelial cells. Exposure of the tissue sections to either preimmune serum (Figs 4B, D) or the anti-*Hom s 1* anti-serum that had been preabsorbed with the recombinant molecule (data not shown) yielded negative results. Similar to what has been reported for IgG-defined autoantigens (Tan, 1991; Nousari and Anhalt, 1995), *Hom s 1* was mainly expressed within the cytoplasm as also demonstrated by immunoelectron-microscopic studies on HepG2 cells (Fig 4E). Interestingly, the anti-*Hom s 1*-reactive product was frequently found

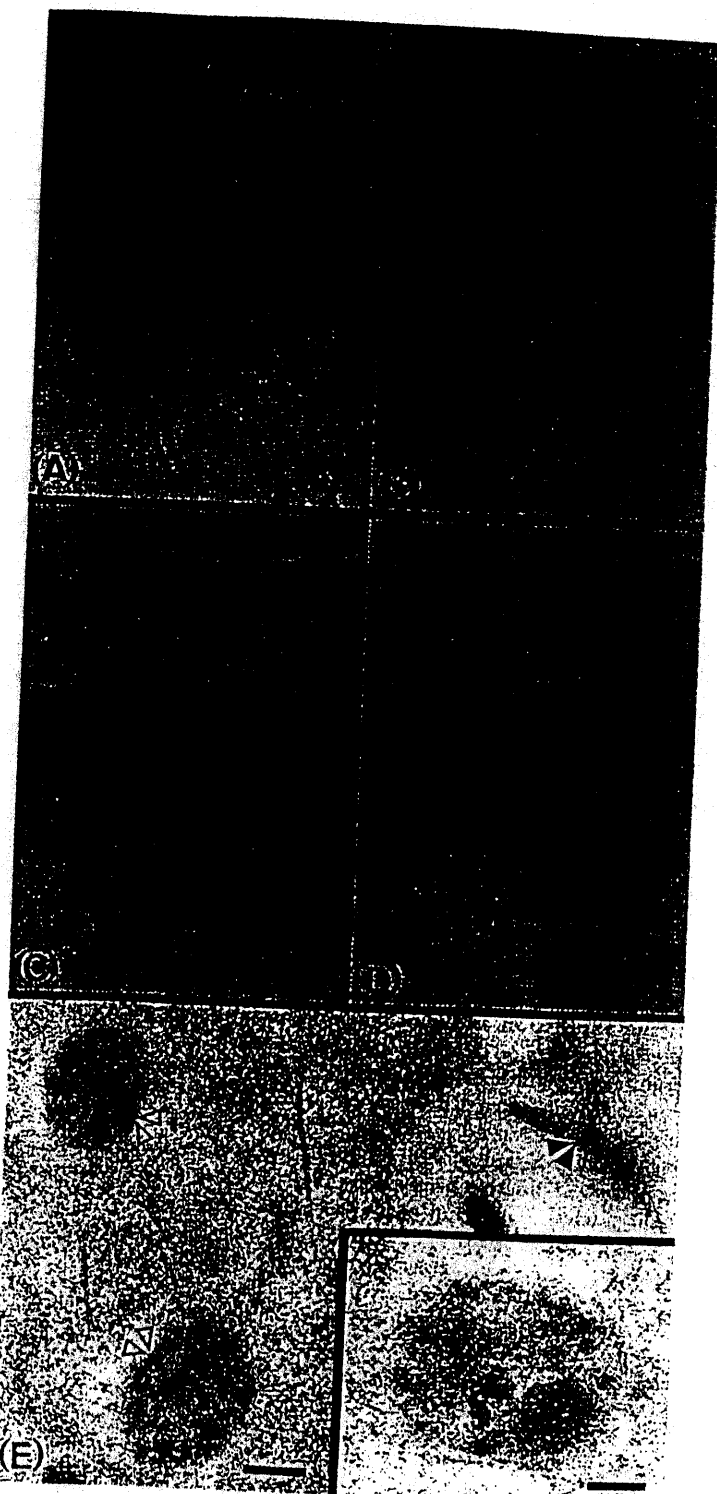


**Figure 4.** *In vivo* expression and ultrastructural localization of *Hom s 1*. Lesional AD (A, B) and normal (C, D) skin sections were stained with either rabbit anti-*Hom s 1* Ig (A, C) or preimmune rabbit Ig (B, D) using the immuno-peroxidase technique described in *Materials and Methods*. (E) For immunoelectronmicroscopic investigations, 80 nm ultrathin sections of HepG2 cells were labeled with anti-*Hom s 1* Ig followed by goat anti-rabbit IgG coupled to 10 nm colloidal gold particles. Labeling was seen within endosomal compartments (e.g., multivesicular bodies; open arrowheads) and was also associated with microvilli (solid arrowheads). A close-up of an *Hom s 1*-containing multivesicular body is shown in the inset. Scale bars: (E) 0.2  $\mu$ m; inset, 0.16  $\mu$ m.

in the endosomal compartment (multivesicular bodies) that serves as a protein/antigen-processing unit in other cell types (Brodsky and Guagliardi, 1991; Germain and Margulies, 1993; Mellman *et al*, 1995). Clustered gold particles were also seen on microvilli protruding from



**Figure 5.** Detection of circulating IgE-*Hom s 1* immune complexes. IgE was affinity purified from sera of two individuals with (DM, SJ) and two without (VR, ZH) detectable *Hom s 1*-specific IgE antibodies. The total Ig levels (kU per liter) in the sera were as follows: DM, 1756; SJ, 2100; VR, 3800; ZH, 3800. Nitrocellulose strips were probed with rabbit anti-*Hom s 1* Ig (lane 1), preimmune Ig (lane 3), rabbit anti-birch profilin (RP3) Ig (lane 2), or an anti-human IgE antibodies (lanes 4).

the cell surface. It was not possible to unequivocally localize the electron-dense immunoreactant to either the outer or the inner layer of the plasma membrane (Fig 4E).

**Detection of circulating *Hom s 1*-IgE complexes in sera of AD patients** Next, we investigated whether *Hom s 1*, besides being cell-bound, can be released into the circulation and, if so, may occur in an IgE-complexed form. IgE was affinity purified from sera of individuals with (DM, SJ) and without (VR, ZH) detectable *Hom s 1*-specific IgE antibodies using CNBr-sepharose-coupled monoclonal anti-human IgE antibodies. Purified IgE fractions were then separated by SDS-PAGE and blotted onto nitrocellulose. Nitrocellulose membranes were probed with the rabbit anti-*Hom s 1* anti-serum and, for control purposes, with preimmune Ig, with a rabbit anti-birch profilin anti-serum (RP3) (Valenta *et al*, 1993) and with anti-human IgG antibodies. Pronounced anti-*Hom s 1* immunoreactivity at 55 kDa was observed in the anti-IgE precipitates from patients DM and SJ, who had displayed IgE reactivity to purified recombinant *Hom s 1* (Fig 5 lane 4). Weakly labeled or no labeled bands of comparable molecular weight were seen in the anti-IgE precipitates of sera from an AD patient without blot-detectable IgE anti-*Hom s 1* (ZH) and from a nonatopic individual (VR) (Fig 5, lane 4). Figure 5 shows that the intensity of anti-*Hom s 1* labeling (lane 4) is not necessarily proportional to the IgE (60 kDa; lane 1) present on the membranes. The preimmune Ig and rabbit anti-birch profilin anti-serum RP3 showed no immunoreactivity with any of the nitrocellulose-blotted anti-IgE precipitates (Fig 5, lanes 3 and 2).

## DISCUSSION

We have recently shown that AD patients frequently and rather selectively display IgE antibody reactivity against human proteins. In contrast, IgG serum antibodies against a wide range of proteins within the very same extracts occurred in many individuals and were not indicative of a particular disease (Valenta *et al*, 1996). Here we report the isolation and molecular characterization of a cDNA coding for one of these IgE-defined autoantigens, designated *Hom s 1*. A cDNA coding for a major portion of *Hom s 1* (clone a) was isolated by immunoscreening of a human epithelial cDNA expression library using serum IgE from AD patients. The *Hom s 1* cDNA and protein sequences greatly resembled those of a recently described antigen (SART-1) recognized by cytotoxic T lymphocytes of a squamous cell carcinoma patient (Shichijo *et al*, 1998).

Similar to the results obtained with cellular extracts (Valenta *et al*, 1996), we found that the  $\beta$ -gal-linked, recombinant *Hom s 1*, although often detected by serum IgG of atopic and nonatopic donors (Valenta *et al*, unpublished observations), reacted prominently with IgE of certain AD patients but not with that of others exhibiting comparable serum IgE levels. Several arguments support the assumption that the immunoreactivity of recombinant *Hom s 1* is representative of the IgE binding pattern of the natural protein. These include the observations (i) that the IgE binding of a recombinant *Hom s 1*-reactive serum to

a 55 kDa epithelial cell-derived protein can be greatly diminished by preabsorbing the very same serum with recombinant *Hom s 1*, and (ii) that a rabbit anti-serum raised against purified recombinant *Hom s 1* strongly reacted with a 55 kDa protein in anti-IgE precipitates obtained from sera of AD patients containing IgE antibodies against recombinant *Hom s 1*. At this point, one should not forget that the molecular weight of SART-1 and that calculated from the first methionine-encoding ATG in the open reading frame of the *Hom s 1* cDNA sequence, should be  $\approx 125$  kDa and 73 kDa, respectively; however, with the exception of muscle-associated *Hom s 1* species (80–85 kDa), the molecular weights of anti-*Hom s 1*-reactive moieties ranged mainly between 46 and 60 kDa. The most likely explanations for the differences between calculated and apparent molecular weights of certain anti-*Hom s 1*-reactive moieties are that *Hom s 1* can be subjected to tissue-specific post-translational modifications and/or degradation events occurring during protein extraction. Neither event would be unexpected considering the localization of *Hom s 1* in the endosomal compartment of the cell.

In view of the reported localization of SART-1 in the nuclei of both normal and malignant cells (Shichijo *et al.*, 1998), we were surprised that our polyclonal anti-*Hom s 1* anti-serum did not exhibit nuclear reactivity by either immunoelectron microscopy, immunohistochemistry (Fig 4), or western blotting (data not shown). One possibility for this discrepancy could be that neither the anti-*Hom s 1* nor the anti-SART-1 anti-serum were raised by immunization with the authentic proteins.

The biologic significance of the IgE anti-*Hom s 1* immune response escapes us at the present time. It may well represent a nonpathogenic bystander phenomenon but, on the other hand, could also play a critical role in the pathogenesis of allergic tissue inflammation in, at least certain patients. In this regard two mutually nonexclusive scenarios are conceivable. First, IgE autoantibodies, resulting from early sensitization events, may bind in their monomeric form to high-affinity IgE receptors present on tissue-bound effector (mast cells, basophils, eosinophils) as well as inducer (monocytes, dendritic cells; Mudde *et al.*, 1990; Maurer *et al.*, 1995, 1996) cells of the allergic response. Autoallergens (e.g., *Hom s 1*), released after tissue injury, may then trigger the signal cascade leading to both immediate-type and delayed-type hypersensitivity reactions seen in the target organs of atopy (i.e., skin, lung, gastrointestinal tract). This assumption is supported by our findings that *Hom s 1* is preferentially expressed in these very same tissues and that the purified recombinant  $\beta$ -gal-fused *Hom s 1*, but not  $\beta$ -gal alone, elicited subtle erythematous skin reactions (patient MD, 6 mm mean diameter; patient SE, 5 mm mean diameter) in two AD patients with *Hom s 1*-specific IgE antibodies.

It is equally possible that the cellular release of autoantigens (e.g., *Hom s 1*) leads to the formation of circulating immune complexes, which, after IgE receptor cross-linking, could induce allergic tissue reactions at organ sites distant from the antigenic source. Evidence for the presence of such immune complexes comes from our finding that, by immunoblotting, a rabbit anti-*Hom s 1* anti-serum detected a 55 kDa moiety in affinity-purified IgE preparations from two *Hom s 1*-reactive donors and, to a much lesser extent, in those from nonreactive persons.

We have previously reported that individuals with IgE antibodies against human proteins frequently suffer from severe cutaneous manifestations of atopy (Valenta *et al.*, 1996). The definition and characterization of the autoantigens involved should therefore allow us to recognize the autoimmune component of the atopic tissue reaction, to monitor disease activity in AD patients, and, more importantly, to develop therapeutic vaccines capable of modulating the allergic autoimmune response.

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