Identification and Immunohistochemical Localization of Protein Precursors to Human Axillary Odors in Apocrine Glands and Secretions

Andrew I. Spielman, DMD, PhD; Gulshan Sunavala, DDS, MS; Judith A. K. Harmony, PhD; William D. Stuart, BS; James J. Leyden, MD; Gloria Turner, BS; Benjamin R. Vowels, PhD; Wan C. Lam, DDS; Shinjun Yang, MD; George Preti, PhD

Objectives: To determine the cellular localization in male and female axillary tissue for apocrine secretion odor-binding proteins 1 (ASOB1) and 2 (ASOB2) and the electrophoretic pattern of female apocrine proteins and to begin characterization of the ASOB1 protein.

Design: Immunohistochemical techniques were used with biopsy samples from axillary tissue of male and female subjects. Immunological techniques and microsequencing were used to characterize several of the proteins in male and female apocrine secretions.

Setting: A university medical center.

Participants: Healthy male and female volunteers who donated apocrine secretions and/or axillary tissue.

Results: Specific immunoreactivity was localized only to the apocrine glands in both sexes. Furthermore, only preabsorption with a mixed apocrine secretion sample eliminated all immunoreactivity. The electrophoretic pattern of proteins in female apocrine secretions is similar to that in male secretions. Western blotting of the separated proteins from female samples using serum samples containing antibodies to ASOB1 and ASOB2 yielded identical results to those found with separated proteins from male samples. Partial sequence data obtained from the N-terminus of ASOB1 suggested that it shares homology with the α-chain of apolipoprotein J (Apo J). Apocrine secretion odor-binding protein 1 is not immunologically similar to Apo J, but 2 other apocrine secretion proteins are.

Conclusions: Male and female subjects appear to have the same glycoprotein carriers for (E)-3-methyl-2-hexenoic acid localized to the apocrine glands. The N-terminal sequence for ASOB1 may be homologous to Apo J, but it is not immunologically similar to it. However, 2 other proteins in the apocrine secretion appear to be the monomer and dimer forms of Apo J.

Arch Dermatol. 1998;134:813-818

MORE THAN 30 years ago, Shehadeh and Kligman1,2 demonstrated that typical body odors were produced by incubating odorless axillary apocrine sweat droplets with resident cutaneous bacteria. Results of early studies suggested that the volatile chemicals responsible for axillary odors were odoriferous steroids and isovaleric acid.3-6 More recently, it has been shown that the structure of characteristic axillary odors consists of C6 to C11, straight-chained, branched, and unsaturated acids.7,8 Analytically, the most abundant of these compounds is (E)-3-methyl-2-hexenoic acid (3M2H), whose odor is very similar to the entire axillary bouquet. Neutral and basic compounds have little to no odor.9,10 The E-isomer of 3-methyl-2-hexenoic acid is present in far greater quantities than volatile odoriferous steroids, although both have low olfactory thresholds.9,11

In further studies, it was determined that 3M2H arrives on the skin surface bound to 2 proteins designated apocrine secretion odor-binding proteins 1 (ASOB1) and 2 (ASOB2), with molecular weights of 45 and 26 kDa, respectively.12 Recently, it was demonstrated that ASOB2 is identical in its polypeptide sequence to apolipoprotein D (Apo D).13 However, its pattern of glycosylation differs from that of Apo D isolated from plasma, demonstrating an apocrine-specific Apo D.

In this study, we used polyclonal antibodies to ASOB1 and ASOB2 to demonstrate the cellular localization of the ASOB proteins to apocrine glands. In addition, partial-sequence data obtained from the N-terminus of ASOB1 suggested that it shared homology with the α-chain of Apo J. However, ASOB1 is not immunologically similar to Apo J.
SUBJECTS AND METHODS

All reagents were obtained from commercial sources and were of analytical grade. The N-terminal sequence for ASOB1 was obtained using microsequencing with Edman degradation by the University of Southern California Microchemical Core Laboratory, Los Angeles, Calif.

All procedures involving human subjects were approved by the institutional review board of the University of Pennsylvania, Philadelphia. Informed consent was obtained from each subject following a detailed explanation of the procedure(s).

Antibodies to ASOB1 and ASOB2 were raised in guinea pigs using 5 to 7 µg each of ASOB1 and ASOB2 purified from a mixture of apocrine secretions from male volunteers as detailed elsewhere.12

Apo J ANTIBODIES

Monoclonal and polyclonal antibodies to Apo J were prepared as previously described.14-16

APOCRINE SECRETION COLLECTION

Apocrine secretions used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting experiments, and protein determination were collected from 7 healthy women (age range, 20-34 years; mean ± SD, 25.4 ± 5.9 years) as previously reported.5-8

BIOPSY SPECIMENS

Biopsy specimens of the underarm area were obtained from healthy male (n = 12) and female (n = 3) volunteers within 1 to 2 minutes following local anesthesia with lidocaine hydrochloride containing epinephrine hydrochloride at a dilution of 1:100 000. Samples were fixed in 4% paraformaldehyde and 0.5% glutaraldehyde in phosphate-buffered saline solution (pH, 7.2) for 4 hours at 4°C. The samples were paraffin-embedded, sectioned at 5 µm, and placed on precoated slides (SuperFrost Plus; Fisher Scientific, Springfield, NJ).

IMMUNOLOGICAL TECHNIQUES

Serum samples containing antibodies to ASOB1 and ASOB2 were partially purified to immunoglobulin fraction using a commercially available kit (E Z SEP; Pharmacia, Piscataway, NJ).

For immunohistochemistry, the serum samples containing antibodies to ASOB1 and ASOB2 or their respective immunoglobulin fractions were used at dilutions ranging from 1:100 to 1:8000. Incubation with the primary antibodies was performed for 1 hour at 22°C. Development of the antibodies was achieved using the avidin-biotin procedure (Vector Laboratories, Burlingame, Calif) with horseradish peroxidase. The slides were counterstained with 0.049% Light Green SF in aqueous solution (Sigma Chemical Company, St Louis, Mo) and mounted.

Control experiments used normal (preimmune) guinea pig serum samples at the same dilution, omission of the primary (specific) antiserum, inclusion of another primary antiserum raised to an unrelated protein (catfish barrel membrane protein)17 using a similar procedure to that used for raising antibodies to ASOB1 or ASOB2, and preabsorption of antibodies with male apocrine secretions collected from either 1 or 5 male donors.

PROTEIN SEPARATION AND WESTERN BLOTTING

Total protein levels in 7 female apocrine samples were determined using the Bradford assay, with bovine serum gamma globulin as a standard.18 Proteins found in the apocrine secretions collected from several female donors (n = 7) were separated using SDS-PAGE as described previously.12,19 Proteins were stained for visualization using Coomassie brilliant blue R-250 or transferred for Western blotting.20

Western blotting was performed according to previously described procedures.12,20 Where monoclonal antibodies to Apo J antibodies were used, conditions were as follows: 10% to 15% SDS-polyacrylamide gels were run under reducing conditions.18 After transfer, primary antibodies were incubated at a dilution of 1:1000, followed by incubation with a peroxidase-conjugated rat anti-mouse IgG and hydrogen peroxide and diamino benzidine hydrochloride as substrates.

RESULTS

Serum samples containing antibodies against ASOB1 and ASOB2 demonstrated specific immunoreactivity, even at dilutions of 1:4000, following immunohistochemistry. There were apparent differences in the staining pattern between male and female biopsy samples. Male samples tended to show more immunoreactive material within the lumen of the apocrine glands and less within the acinar structure (Figure 1, A and B) than female samples (Figure 1, C and D). Females demonstrated more immunoreactivity in the acinar structures than did samples from male structures.

None of the control slides demonstrated specific reactivity. Figure 1 demonstrates that eccrine and sebaceous glands and hair follicles did not contain any specific immunoreactivity. Localization to the apocrine glands was highly specific and concentration dependent. In addition, there was no consistent difference between the immunoreactive pattern of ASOB1 and ASOB2.

Two types of preabsorption experiments were performed. The first used apocrine secretion from a single male donor to interact with the antibodies to ASOB1 or ASOB2 before incubation with antisera to the axillary tissue. This resulted in a noticeable drop in specific immunoreactivity, but some residual reaction could still be seen within the apical region of the acini. The second used mixed apocrine secretions from 5 male donors preincubated with antibodies to ASOB1 or ASOB2 before incubation with axillary tissues. This procedure eliminated immunoreactivity in the tissue samples. Data obtained from performing the same experiments with anti-ASOB1 yielded similar results (data not shown).

The apocrine secretions from 7 of the female donors were assayed for total protein content.18 The average amount of protein and the variation found in our fe-
male subjects are identical to those previously reported in male apocrine secretions (mean ± SD levels in female samples, 4.26 ± 3.22 g/L; in male samples [n = 10], 4.22 ± 3.54 g/L).

Proteins found in the apocrine secretions of 5 female donors were electrophoretically separated using SDS-PAGE. These profiles (data not shown) were qualitatively similar to the electrophoretic profiles obtained from male donors.12 In addition to ASOB1 and ASOB2 seen at apparent molecular masses of 45 and 26 kd, respectively, other prominent bands are seen at apparent molecular masses of 40 and 66 kd.

Serum samples containing antibodies to ASOB1 and ASOB2 were used to examine these separated proteins from female donors following Western blotting. The results, shown in Figure 2, were identical to those found when similarly separated samples from male donors were probed with these antisera12; ie, antiserum to ASOB2 reacted only with the protein at apparent molecular mass of 26 kd (ASOB2), whereas antiserum to ASOB1 cross-reacted with the proteins at apparent molecular masses 26 and 45 kd (ASOB1).

Further characterization of ASOB1 was performed using 2 independently isolated samples (approximately 1-2 nmol each) isolated from 6 to 8 male donors. Two N-terminal sequences were obtained; both agreed well with each other as shown in the Table. A homology search of these sequences was performed using protein sequences in GenBank (NCBI, Bethesda, Md). This search identified the N-terminal sequence of the α-chain from Apo J14,16 as being highly homologous to the N-terminus of ASOB1. The N-terminal sequence of Apo J (first 15 amino acids) and ASOB1 (2 determinations) are listed in the Table. The first 12 amino acid residues in the N-terminus of ASOB1 and the α-chain of Apo J14 share 7 residues; 9, if conservative substitution is allowed (Table).

Apolipoprotein J is a 70-kd glycoprotein consisting of 2 disulfide-linked units (chains) designated as the α-chain (34-36 kd) and β-chain (36-39 kd). Both chains are derived from a single polypeptide precursor using proteolysis between amino acid residues 204 and 205. In plasma, Apo J is found in association with high-density lipoproteins.14,16

To determine if Apo J and ASOB1 were immunologically related, the apocrine secretion proteins from 1 donor were separated using SDS-PAGE and probed with monoclonal and polyclonal antibodies raised against Apo J. The ASOB1 did not cross-react with either of these antibodies; further, serum samples containing antibodies to ASOB1 did not react with human serum Apo J when tested using Western blotting under reducing conditions. However, 2 other proteins in apocrine secretions,
Apo J, a behavior reminiscent of serum Apo J.16

4 proteins comigrated with purified 66- and 40-kd apocrine proteins (Figure 3, lanes 2 and 5), and 3 and 6 each contained samples from the same subjects. The apparent molecular weight of the proteins (in kilodaltons) is shown on the left. On the right, 1 and 2 identify ASOB1 and ASOB2, respectively. Specific immunoreactivity of antibodies to ASOB2 with the 26-kd ASOB2 is seen, whereas antibodies to ASOB1 reacted with ASOB1 and ASOB2.

Figure 3. Western blotting of apocrine protein sepa-
ated under nonreducing conditions demonstrated a 66-kd protein that reacted with antibodies to Apo J and an immunoreactive smear at higher molecular mass, suggestive of higher aggregates (ie, trimers, tetramers) of Apo J (Figure 3, lane 1). The same sample under reducing conditions demonstrated 2 immunoreactive proteins migrating at 66 and 40 kd (Figure 3, lanes 2 and 5). The 66-kd band in lane 5 is present albeit far weaker than in lane 2, suggesting more complete reduction of the sample analyzed in lane 5 or less uncleaved native Apo J. These proteins comigrated with purified 66- and 40-kd apocrine proteins (Figure 3, lanes 3 and 4). Pure 40-kd protein, isolated using electroelution from SDS-PAGE (Figure 3, lane 4), forms immunoreactive dimers and possibly tetramers, a behavior reminiscent of serum Apo J.16

Monoclonal anti–Apo J antibody14 was also used to locate Apo J in axillary tissue. This showed a weak but distinct immunoreactivity only in the cells surrounding the apocrine gland lumen and material within the lumen (1:500 dilution; data not shown). When purified serum Apo J underwent Western blotting and was probed with anti–Apo J (Figure 3, lane 6), 2 proteins were visible migrating at 71 and 35 kd.

Although it has been assumed for more than 30 years3,10,11 that the odorless precursors to axillary odor reside within the apocrine glands, data presented herein demonstrate this directly. Only axillary apocrine glands from male and female subjects demonstrated immunoreactivity to serum samples containing antibodies raised to ASOB1 and ASOB2. No immunoreactivity was seen in any other glandular structures or supporting tissue found in the axillae (Figure 1).

The pattern of electrophoretically separated proteins from epinephrine-stimulated apocrine secretions of women appeared almost identical to that previously reported for men.12 Probing the separated proteins from women with serum samples containing antibodies to ASOB1 and ASOB2 revealed 2 proteins at apparent molecular masses of 45 and 26 kd, respectively. Consequently, serum samples containing antibodies raised against male ASOB1 and ASOB2 appear to recognize the same antigenic determinants in female ASOB1 and ASOB2. Therefore, it appears that similar axillary odor-carrier proteins reside in the underarms of both sexes. However, it is not possible to judge from the present data whether the 3M2H is bound to the proteins in tissue samples.

The results from the preabsorption experiments could be traced back to the procedure used to generate the antibodies to ASOB1 and ASOB2. These were raised against a mixture of purified ASOB1 and ASOB2 collected from several male donors.12 Purified ASOB1 and ASOB2 appeared homogeneous using SDS-PAGE before their use as antigens. However, although a previous study revealed no polymorphism in the polypeptide chain of ASOB2,13 individual variation in glycosylation patterns at antigenic sites could not be ruled out. Consequently, an individual would not supply a diverse enough antigen array or sufficient antigen to preabsorb all antibodies.

An unexpected, albeit positive, finding in our study resulted from probing apocrine secretion proteins with antibodies to Apo J, demonstrating that intact Apo J and one or both of its α- and β-chains are present (Figure 3). However, the apocrine–Apo J protein does not appear to be identical to serum Apo J, due to their slightly different migration when separated by SDS-PAGE. The subunits of serum Apo J migrate at 35 to 40 kd under reducing conditions. The Apo J subunits are derived using proteolysis of a single amino acid chain. However, approximately 5% of Apo J is not cleaved in plasma,14 and it exists as a disulfide cross-linked monomer of 70 kd. Also, depending on the reducing conditions used (especially if pH < 7.0), some Apo J will not be reduced completely.15,16 We employed a pH of 6.8 during reduction.19

COMMENT

ARCH DERMATOL / VOL 134, JULY 1998

816

©1998 American Medical Association. All rights reserved.

Downloaded From: http://archderm.jamanetwork.com/pdfaccess.ashx?url=/data/journals/derm/4613/ on 04/02/2017
Evidence presented herein demonstrates that the properties of the proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.

Male and female tissue samples displayed different patterns of immunoreactivity for the ASOB proteins. Male apocrine glands possessed greater amounts of immunoreactive proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens.

Finally, since the monoclonal antibody was raised against intact (nonreduced) Apo J, it reacts preferentially toward the uncleaved or unreduced form. Western blotting results can therefore be skewed to make it appear that much more Apo J is present as the 70-kd form (under reducing conditions) than is visible with Coomassie stain.

Previous studies suggest that the proteins at apparent molecular masses of 40 and 66 kd do not have 3M2H associated with them. Although other volatile compounds may be bound to these proteins in apocrine secretion, we have not identified them yet.

A recent study has described the qualitative similarity between male and female axillary odor components and has determined that their precursors are aqueous-soluble molecules found in female apocrine secretions. Evidence presented herein demonstrates that 2 proteins found in female apocrine secretions with apparent molecular weight of 26 and 45 kd are immunologically similar to the ASOB1 and ASOB2 found in male secretions. Further, the total amount of protein present in the female apocrine secretions and the pattern of proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.

Male and female tissue samples displayed different patterns of immunoreactivity for the ASOB proteins. Male apocrine glands possessed greater amounts of immunoreactive proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens.

Finally, since the monoclonal antibody was raised against intact (nonreduced) Apo J, it reacts preferentially toward the uncleaved or unreduced form. Western blotting results can therefore be skewed to make it appear that much more Apo J is present as the 70-kd form (under reducing conditions) than is visible with Coomassie stain.

Previous studies suggest that the proteins at apparent molecular masses of 40 and 66 kd do not have 3M2H associated with them. Although other volatile compounds may be bound to these proteins in apocrine secretion, we have not identified them yet.

A recent study has described the qualitative similarity between male and female axillary odor components and has determined that their precursors are aqueous-soluble molecules found in female apocrine secretions. Evidence presented herein demonstrates that 2 proteins found in female apocrine secretions with apparent molecular weight of 26 and 45 kd are immunologically similar to the ASOB1 and ASOB2 found in male secretions. Further, the total amount of protein present in the female apocrine secretions and the pattern of proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.

Male and female tissue samples displayed different patterns of immunoreactivity for the ASOB proteins. Male apocrine glands possessed greater amounts of immunoreactive proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens.

Finally, since the monoclonal antibody was raised against intact (nonreduced) Apo J, it reacts preferentially toward the uncleaved or unreduced form. Western blotting results can therefore be skewed to make it appear that much more Apo J is present as the 70-kd form (under reducing conditions) than is visible with Coomassie stain.

Previous studies suggest that the proteins at apparent molecular masses of 40 and 66 kd do not have 3M2H associated with them. Although other volatile compounds may be bound to these proteins in apocrine secretion, we have not identified them yet.

A recent study has described the qualitative similarity between male and female axillary odor components and has determined that their precursors are aqueous-soluble molecules found in female apocrine secretions. Evidence presented herein demonstrates that 2 proteins found in female apocrine secretions with apparent molecular weight of 26 and 45 kd are immunologically similar to the ASOB1 and ASOB2 found in male secretions. Further, the total amount of protein present in the female apocrine secretions and the pattern of proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.

Male and female tissue samples displayed different patterns of immunoreactivity for the ASOB proteins. Male apocrine glands possessed greater amounts of immunoreactive proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens.

Finally, since the monoclonal antibody was raised against intact (nonreduced) Apo J, it reacts preferentially toward the uncleaved or unreduced form. Western blotting results can therefore be skewed to make it appear that much more Apo J is present as the 70-kd form (under reducing conditions) than is visible with Coomassie stain.

Previous studies suggest that the proteins at apparent molecular masses of 40 and 66 kd do not have 3M2H associated with them. Although other volatile compounds may be bound to these proteins in apocrine secretion, we have not identified them yet.

A recent study has described the qualitative similarity between male and female axillary odor components and has determined that their precursors are aqueous-soluble molecules found in female apocrine secretions. Evidence presented herein demonstrates that 2 proteins found in female apocrine secretions with apparent molecular weight of 26 and 45 kd are immunologically similar to the ASOB1 and ASOB2 found in male secretions. Further, the total amount of protein present in the female apocrine secretions and the pattern of proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.

Male and female tissue samples displayed different patterns of immunoreactivity for the ASOB proteins. Male apocrine glands possessed greater amounts of immunoreactive proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens. Since apocrine glands are androgen target organs, male apocrine glands may contain more proteins in their lumens.

Finally, since the monoclonal antibody was raised against intact (nonreduced) Apo J, it reacts preferentially toward the uncleaved or unreduced form. Western blotting results can therefore be skewed to make it appear that much more Apo J is present as the 70-kd form (under reducing conditions) than is visible with Coomassie stain.

Previous studies suggest that the proteins at apparent molecular masses of 40 and 66 kd do not have 3M2H associated with them. Although other volatile compounds may be bound to these proteins in apocrine secretion, we have not identified them yet.

A recent study has described the qualitative similarity between male and female axillary odor components and has determined that their precursors are aqueous-soluble molecules found in female apocrine secretions. Evidence presented herein demonstrates that 2 proteins found in female apocrine secretions with apparent molecular weight of 26 and 45 kd are immunologically similar to the ASOB1 and ASOB2 found in male secretions. Further, the total amount of protein present in the female apocrine secretions and the pattern of proteins seen using SDS-PAGE are similar with respect to the major constituents (at apparent molecular masses of 26, 40, 45, and 66 kd). The presence of proteins at 26 and 45 kd in the female apocrine secretions and their immunoreactivity with serum samples containing antibodies to ASOB1 and ASOB2 when combined with the chemistry data cited above suggest that 3M2H is also likely bound to these proteins in female apocrine secretions. Consequently, there is a high degree of qualitative similarity for each sex in the odors produced and the precursor-carrier proteins to one of the major odorants.
REFERENCES