This study examines the distribution of A, B, and H substances in 15 syringomas and 11 control axillary skins. Monoclonal antibodies (MCABs) against A, B, and H substances with the avidin–biotin–peroxidase complex (ABC) method were used. Additionally, the H antigen was localized with *Ulex europaeus* Agglutinin-I (UEA-I) by use of the peroxidase–antiperoxidase method. The MCABs provided specific and reproducible staining of all 15 syringomas as well as the secretory coil and duct of normal eccrine glands. Contrariwise, apocrine and sebaceous glands were uniformly nonreactive. The staining by the UEA-I technique showed a similar pattern to that of the anti-H MCAB but with increased background (nonspecific) staining. These results, using state-of-the-art immunohistochemistry, confirm the eccrine origin of syringoma. Blood group substances may be useful in distinguishing other tumors of eccrine origin from those of apocrine origin. (Key words: Syringoma; Adnexal tumors; Blood group substances; Immunohistochemistry) Am J Clin Pathol 1988;89:778–783

ADNEXAL TUMORS of the skin continue to confuse and frustrate pathologists. The neoplasms are rare, and therefore systematic review of large numbers of tumors is lacking. Consequently, adnexal neoplasms remain shrouded in complexities of nomenclature and ambiguities of histogenesis. Along with the advent of immunologic techniques applied to tissue has come a better understanding of the biologic features of skin, its adnexa, and adnexal-derived neoplasms.

The A, B, and H substances are glycoproteins present not only in the membranes of erythrocytes, but also in many different epithelia. Various immunologic tools have been used to demonstrate these substances in the epidermis and adnexal structures. Of special note is the presence of substances A, B, or H in eccrine and eccrine-derived tumors but not apocrine or sebaceous sweat glands, as determined by the red blood cell adherence (RCA) test. With the advent of monoclonal antibodies came precise immunologic probes readily available to the pathologist. Because these tools can aid in classifying neoplasms as well as elucidating their histogenesis, we thought it important to further evaluate the immunogenicity of adnexal tumors, specifically syringoma, and normal sweat glands. Using monoclonal antibodies with the avidin–biotin–peroxidase complex (ABC) technique and a lectin with the peroxidase–antiperoxidase (PAP) procedure, we found syringomas to be consistently immunoreactive for blood group substances, as are the eccrine glands but not other sweat glands.

**Materials and Methods**

Formalin-fixed paraffin-embedded skin was available in 15 cases of syringoma and 11 cases of normal axillary tissue removed at autopsy. Serial 5-μm-thick sections of tumor were stained with hematoxylin and eosin, and other replicate sections were subjected to immunoperoxidase. Briefly, deparaffinized sections of tumor were treated with 0.3% (v/v) hydrogen peroxide in methanol for 30 minutes to block endogenous peroxide activity. The sections were then rehydrated and digested with 0.1% (w/v) trypsin in 0.1% (w/v) calcium chloride (0.1 g of trypsin and 0.1 g of calcium chloride diluted to 100 mL with distilled water in a 100-mL volumetric flask) brought to pH 7.8 with 0.1 mol/L tris (Sigma) to 900 mL of distilled water plus 8.8 g sodium.
chlorin-labeled horse antimouse IgG was added for 20 minutes, followed by the ABC technique (Stravinger® Bio- 
in Strept Avidin ImmunoStaining Kit, Biogenex Laboratories, Burlington, CA) for 20 minutes at room 
temperature.

Sites of peroxidase activity were visualized with 0.025% dianinobenzidine with 0.009% (u/v) hydrogen 
peroxide in 0.05 mmol/L TRIS buffer (formula as above, but without sodium chloride), pH 7.6. The sec-
tions were counterstained with Harris’ hematoxylin and coverslipped.

In addition, 11 of the 15 syringoma cases and all the 
axillary skin excisions were evaluated with Ulex eu-
ropaecus Agglutinin-I (UEA-I) by the unlabeled antibody 
PAP method of Sternberger.15 Incubation of the sections 
with 10% (v/v) normal sheep serum for 10 minutes was 
followed by sequential application of UEA-I, diluted 
1:100, for two hours at room temperature and a poly-
clonal goat anti-UEA-I (1:100, Vector Laboratories, 
Burlington, CA) primary antibody for 18 hours at -4 
°C. After rinsing with TBS, a sheep antigoat antibody 
(1:20, Cappel Laboratories, Cochranville, PA) was ap-
plied for 30 minutes. The final immunoreactant was 
goat PAP (1:200, Dako, Santa Barbara, CA).

The specificity of the immunostaining was verified by 
use of a positive control (pancreas) and by replacing 
the primary antisera with nonimmune serum of compara-
bale dilution from the same species from which the pri-
mary antibody was produced. In addition, the ubiquity 
of blood-filled vessels and intimal lining of small blood 
vessels in the dermis and subcutaneous fat, along with 
the inner root sheath of the hair follicles and immuno-
positive stratum corneum, served as built-in positive 
controls. The basal layer, dermis, and subcutaneous fat 
were nonreactive and served as internal negative con-
trols. Usually at least two serial sections were on each 
slide, thereby duplicating the immunoreaction. The epi-
dermis and adnexa were evaluated for immunoreactiv-
ity and graded as negative, mild (scattered positive cells), 
moderate (most cells weakly positive), or marked (most 
cells strongly positive).

Results

Histologic Findings

The diagnosis of syringoma was based on well-es-
ablished histologic criteria, including numerous small 
ducts embedded in a fibrous stroma, the walls of which 
were lined by two rows of epithelial cells.11 The latter 
were flat or vacuolated. The lumina of the ducts con-
tained amorphous or keratinous debris, and some of the 
ducts possessed small comma-like tails of epithelial cells. 
Strands of neoplastic basophilic epithelial cells indepen-
dent of the ducts were also seen (Figs. 1 and 2). The 
acrosyringium, duct, and secretory coil of the eccrine 
glands as well as apocrine glands were readily identified 
in adjacent nontumorous tissue and in the axillary con-
trol tissues.

Immunohistochemistry

All syringomas demonstrated reactivity for the blood 
group substances and UEA-I (Fig. 2). The immunoreac-
tivity varied from tumor to tumor as well as from field to field within a single tumor, depending on the mono-
clonal antibody applied. In general, immunoreactivity 
was stronger with anti-B than with the other antibodies.

However, most tumor cells, regardless of type of anti-
body, stained strongly along their cell membranes, most 
notably at the luminal surfaces, whereas cytoplasmic 
staining was mild to negative. Thirteen of the 15 tumors 
had intraluminal secretions; the secretions were more 
immunoreactive than the individual tumor cells in all 
13 cases (Fig. 2). Although our numbers are small, the 
distribution of blood groups in the tumors did not differ 
makedly from that in the general population: seven, 
six, and two cases were blood groups O, A, and B, re-
spectively. All seven syringomas of blood group O ex-
pressed the H substance but lacked the A and B sub-
stances by monoclonal antibodies. UEA-I staining was 
performed on six of the seven cases and the reaction was 
comparable to the monoclonal antibody for the H sub-
stance, although less intense. Also, the UEA-I had mild 
nonspecific “background” staining of connective tissues 
not present with the monoclonal antibodies.

All syringomas that expressed the blood group A or B 
substances coexpressed H as well as lectin reactivity. 
Again, the latter immunoreactivity was mild (scattered 
positive cells).

Finally, in addition to tumor positivity, we noted that 
the eccrine sweat apparatus in all 11 control cases 
marked consistently for blood group substances (Fig. 2, 
inset). The degree of positivity varied within the appa-
ratus from moderate (most cells weakly positive) in the 
secretory coil to marked (most cells strongly positive) in 
the eccrine duct. The acrosyringium also had positive 
results but with a weaker reaction than that seen in the 
underlying intradermal duct. As observed in the syrin-
gomas, immunoreactivity was maximal with anti-B and 
less intense with the other immunoreactants. Although 
the eccrine sweat gland adnexa consistently stained for 
blood group substances, apocrine and sebaceous glands 
uniformly had negative results (Fig. 3).

Discussion

The study of the immunobiology of skin is unraveling 
at a feverish pace.2-4,9,12,13,16,19,21 Why blood group sub-
FIG. 1. Syringoma characterized by numerous debris-filled cystic ducts of varying size and shape embedded in fibrous stroma of the dermis. Hematoxylin and eosin (X63).

stances are present in skin is not known. These glyco-
proteins can readily be detected with a variety of immu-
nologic methods. Using the RCA method, workers re-
ported the selective distribution of blood group sub-
stances in eccrine but not apocrine or sebaceous
 glands. Additionally, these immunomarkers were
found in four syringomas, and it was therefore suggested
that this tumor was of eccrine origin. With the advent of
monoclonal antibodies the RCA has been replaced by
simplified immunoenzymatic procedures. These new
immunohistochemical methods provide better sensitiv-
ity and allow for a greater array of antigens to be evalu-
ated. Monoclonal antibodies, when coupled with the
ABC technique, are highly specific and provide a well-
defined, semiquantitative, localized staining and perma-
nent record of the immunoreaction. Our findings with the use of monoclonal antibodies are in keeping with those previously reported in that all syringomas tested contained blood group substances. The immunolocalization of these substances to the cell
membranes (especially near the luminal surfaces), coexpression of H along with A or B substances, and variability of the staining pattern within both tumors and normal adnexal glands can be explained by the intracytoplasmic synthesis of the precursor H antigen glycoprotein and addition of carbohydrate moieties to produce A and B antigens that eventually become incorporated into the surface cell membrane. Furthermore, expression of the A, B, and H substances on skin appears to be independent of the secretory status of the patient. However, staining intensity is a measure of antigen density, which is dependent on genetic considerations such as heterogeneity of antigen (A/H, B/H) or expression of weak subgroups of blood type A or B (A2, A3, A2B).14,20

Finally, expression of substances is affected by fixation and processing of tissue, which has greater impact on those surface antigens of low density.12,18

In addition to the syringoma, we found that the eccrine sweat apparatus was also immunoreactive, in keeping with the experience of others.3,12,17,18 However, the absence of immunoreactivity in sebaceous and apocrine glands was notable. This was a consistent and reproducible finding in all of our axillary skin controls (Fig. 3). Recently, Penneys commented that the blood group substances A, B, and H can be demonstrated immunohistochemically within eccrine and apocrine gland epithelium.12 Yet in his review of the immunohistochemistry of adnexal neoplasms, he offers no data or microphotographs demonstrating apocrine reactivity. In our experience, eccrine glands are immunoreactive, whereas other sweat glands completely lack blood group substances or the substances are of insufficient quantity to be readily detected by the ABC technique.

As diagnosticians, can we capitalize on the immunospecificity of sweat glands? Because most benign neoplasms probably maintain immunologic fidelity to their
cell of origin, syringomas have kinship to the eccrine sweat gland because both express blood group substances. The tumor already has been established as an eccrine-derived neoplasm both histochemically and ultrastructurally.5,6,8,21 We now add a third modality, immunocytochemistry, which provides additional evidence that syringomas are differentiated eccrine neoplasms. Monoclonal antibodies, not only to blood group substances but to a variety of epitopes expressed in the adnexa, may ultimately “firm up” the diagnostic quagmire surrounding adnexal tumors of the skin.

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References


Thirteen cases of idiopathic chronic lymphocytosis are the subject of this report. Patients showed a lymphocyte count between 4 and 15 × 10⁹/L for at least six months, marrow lymphocytosis not exceeding 25%, absence of lymphomegaly and hepatosplenomegaly, and no associated infectious, immune, or neoplastic disease. Morphologic examination of smears revealed a lymphocytosis of large granular lymphocytes in five. A selected battery of polyclonal and monoclonal antisera to antigens commonly found on B-, T-, and natural killer cells allowed the identification of six cases of early B-cell chronic lymphocytic leukemia, two cases of T-cell lymphocytosis with a suppressor or helper T-cell phenotype, and five cases of large granular lymphocyte/natural killer cell proliferative disease. The results demonstrate the usefulness of combining morphologic and phenotype studies for the investigation of chronic lymphocytosis, which often appears as an early leukemia or a benign clonal proliferative disorder of lymphocyte subsets. (Key words: Lymphocyte subsets; Lymphocytosis) Am J Clin Pathol 1988;89:783–787

PERSISTENT LYMPHOCYTOSIS in adults is a relatively rare event in the clinical practice, requiring a careful evaluation to assess the existence of a hypothetic underlying leukemic disorder. Adults with chronic lymphocytosis (CL) show an absolute lymphocyte count of 4–15 × 10⁹/L for a minimum of six months, no evidence of lymphadenopathy or hepatosplenomegaly, a bone marrow lymphocytic infiltration below 30%, and no associated infectious, dysimmune, neoplastic, or stress-related condition.4,6,22 Because the minimum requirements for a clinical diagnosis of chronic lymphocytic leukemia include a lymphocytosis of greater than 15 × 10⁹/L and marrow infiltration of at least 30%,7,16 an investigational approach seems necessary to clarify the nature of the expanded lymphocytes in the case of an apparently idiopathic chronic lymphocytosis.

Blood lymphocytes from normal persons constitute a heterogeneous set of cells differing as to morphologic characteristics, immunophenotype, and functional capabilities. These are B-, and T-, and natural killer (NK) cells, the latter exhibiting the large granular lymphocyte (LGL) morphologic characteristics and endowed with spontaneous cytotoxic capacity.9,23

On the assumption that lymphocytes from chronic lymphoproliferative disorders retain cell features of a known normal counterpart, we investigated 13 CL cases with a battery of selected B-, T-, and NK cell immune reagents together with a careful evaluation of cell morphologic characteristics.

Patients and Methods

CL, defined according to the above criteria,4,6,22 was diagnosed in 13 adults (8 men, 5 women): median age 54 years, range 27–80. Absolute lymphocytosis had been observed for 7–47 months and confirmed on at least