Decrease in Le\(^\text{a}\) Expression in Esophageal Adenocarcinomas Arising in
Barrett’s Epithelium\(^1\)

Ulla Engel,\(^2\) Ronda McCombs, Patricia Stranahan,
David Pettijohn,\(^3\) and Esther Hage

State University Hospital, Copenhagen, Denmark [U.E., E.H.], and University
of Colorado Cancer Center, Denver, Colorado 80262 [R. M., P. S., D. P.]

Abstract

Fifty esophageal adenocarcinomas were investigated for their
expression of Le\(^\text{a}\), Le\(^\text{b}\), and Le\(^{\text{a}}-\text{Le}^{\text{b}}\). Among the 50
adenocarcinomas, 17 cases developed in Barrett’s
epithelium. Those 17 differed from the other 33 cases by
expressing much less Le\(^\text{a}\). Fifty-nine percent of Barrett’s
adenocarcinomas were Le\(^\text{a}\) negative compared with 24% of
the non-Barrett’s carcinomas. All Barrett’s adenocarcinomas showed less than 50% Le\(^\text{a}\) whereas
50% of non-Barrett’s carcinomas showed between 50 and
100% expression. The statistical correlation coefficient
for this association was \(P < 0.001\). Normal gastric cardia
epithelium showed the same Le\(^\text{a}\) expression in both
groups. In the Barrett group, Le\(^\text{a}\) expression decreased
from normal through intestinal metaplasia and dysplasia
to adenocarcinoma. This progression was not seen in the
non-Barrett group. Loss of Le\(^\text{a}\) expression may prove
useful in following patients with Barrett’s epithelium in
evaluating progression toward a malignant process. No
difference in expression of Le\(^\text{b}\) and Le\(^{\text{a}}-\text{Le}^{\text{b}}\) was found
between Barrett’s and non-Barrett’s carcinomas.

Introduction

Carcinogenesis has been described as a molecular disease of
cell membrane glycoconjugates (1, 2). Studies have demonstrated
that although cell surface glycosylation varies with cell
and tissue type, changes in relation to transformation have some
common themes. These appear to be either incomplete oligo-
saccharide synthesis or neosynthesis (3–9).

Studies in one of our laboratories have focused on aberrant
glycosylation of mucins and other large glycoproteins in non-
small cell carcinomas of the lung and cancers of the gastroin-
testinal tract (10–12). Initially, we described expression of a
previously unidentified extended Lewis antigen (Le\(^{\text{a}}-\text{Le}^{\text{b}}\)) in
squamous cell lung carcinoma which is recognized by Mab\(^4\)
43-9F. Le\(^{\text{a}}-\text{Le}^{\text{b}}\) is suspected to be exclusively associated with
glycoproteins in contrast to other Lewis antigens which are
glycoprotein/glycolipid carbohydrate moieties (13). These
studies were extended to include the use of additional Mabs to
identify expression of other Lewis antigens which have each,
individually, been described as tumor-associated carbohydrate
antigens by others (1, 14–17). Utilization of a panel of biomarkers
on patient specimen was initiated in each case to increase the
probability of concordant aberrant glycosylation which
would lead to patterns which might have diagnostic and/or
prognostic significance. Furthermore, emerging patterns could
alter patient management decisions.

The current study focuses on adenocarcinomas developing
at the gastroesophageal junction, either in Barrett’s epithelium
(which is columnar epithelium located in the distal esophagus
generally believed to occur secondary to chronic reflux) or in
non-Barrett’s gastric cardia mucosa. Several investigations
have shown that Barrett’s epithelium is premalignant. These
columnar epithelial cells transform first into dysplastic cells
and then, in many instances, progress into adenocarcinomas (16–
18).

We utilized Mabs against Le\(^{\text{a}}-\text{Le}^{\text{b}}\), Le\(^\text{a}\), and Le\(^\text{b}\) to assess
expression in premalignant epithelium and within the adenocarcinomas arising in both gastric cardia mucosa and Barrett’s
epithelium.

Materials and Methods

Tumor Specimens. Slides from 50 esophageal adenocarcino-
mas (41 men and 9 women, ages 20–82 years) were obtained
from the Department of Pathology, State University Hospital,
Copenhagen Hospital Cooperation. The material consists of 17
adenocarcinomas developed in Barrett’s epithelium and 33
other gastric cardia adenocarcinomas (1979–1990). All ade-
nocarcinomas were surgical specimens. The diagnosis of esopha-
geal adenocarcinomas was made if more than half of the
tumor’s length was above the gastroesophageal junction. The
diagnoses of Barrett’s esophagus in the 17 cases were based on
the presence of Barrett’s epithelium proximal to the gastro-
esophageal junction. From each surgical specimen, 15–30 blocks
were taken representing resection lines, nontumorous mucosa,
and pathological mucosa, whereas 1–10 blocks were taken of
tumor and surrounding tissue. From each surgical specimen,
one to six sections of nontumorous gastric cardia and one to
two slides were chosen as representative. From the 17 Barrett
cases, areas of Barrett’s epithelium with intestinal metaplasia
and, if present, dysplasia were chosen as well. Tumor differ-
entiation in both groups included adenocarcinomas which were
poorly, moderately, or well differentiated.

Received 6/21/96; revised 12/23/96; accepted 1/6/97.

\(^1\) This research was supported in part by funding from Onkologisk Forsknings-
seten, State University Hospital, Copenhagen Hospital Cooperation, Denmark
and by funding from Architect Holger Hjortenberg and wife Dagmar Hjortenberg
and by DOD AIBS 2549, P50CA58187 from the U.S. National Cancer Institute
and Howard Hughes Medical Institute Grant 71109-500802 to the University of
Colorado-Hughes Undergraduate Biological Sciences Educational Initiative.

\(^2\) Present address: Nakskovkej 108, 2500 Valby, Denmark.

\(^3\) To whom requests for reprints should be addressed, at University of Colorado
Cancer Center, Box B188, 4200 East 9th Avenue, Denver, CO 80262.

\(^4\) The abbreviation used is: Mab, monoclonal antibody.
Antibodies. The three monoclonal antibodies recognizing Le\(^{a}\) (Co-514), Le\(^{a}\) [P12]), and Le\(^{a}\)-Le\(^{x}\) (43-9F) were individually applied to each section, and both immunofluorescence and immunoperoxidase staining were performed on serial sections from each case. Each Mab was purified from serum-free culture media (RPMI 1640) of the respective hybridomas as described previously (6, 19). In some experiments, the antibody-containing media were used without purification. Purified Mabs were applied at a concentration of about 2 pg/ml (diluted in PBS), whereas Mabs in serum-free culture media were applied after dilution of 1:40 (in PBS).

For immunofluorescence, each tissue section was incubated with primary antibody at room temperature for 1 h in a humidity chamber. After 1 h the tissues were vigorously washed three times with PBS and secondary antibody was applied. For immunofluorescence, the secondary antibody was FITC- or tetramethylrhodamine isothiocyanate-conjugated goat anti-mouse polyclonal IgG/IgM applied at a concentration of 1 \(\mu\)g/ml (Sigma, St. Louis, MO). Again, tissues were incubated for 1 h in humidity chambers at room temperature. After incubation, the tissues were thoroughly rinsed in PBS and several drops of antifade coverslip mounting media were applied to the sections, and the slides were coverslipped and stored in the dark at 4°C until they were evaluated.

For the immunoperoxidase technique, slides were preincubated at room temperature with PBS and BSA (15 min). Primary antibody was then applied and the sections were incubated overnight at 4°C. The peroxidase-conjugated secondary antibody, P260 (DAKO), was then applied in a dilution of 1:20, and the sections were incubated at room temperature for 60 min, developed in 0.04% 3-amino-9-ethylcarbazol for 10 min (Sigma), and counterstained with hematoxylin for 2 min. Aquamount coverslip media were applied and the sections were coverslipped.

On a routine basis, H&E sections were obtained adjacent to those sections which were evaluated using both immunohistochemistry methods.

Evaluation. Evaluation of the patient specimen was on the basis of the fraction of positively stained tumor cells as well as the fraction of positively stained dysplastic or nondysplastic Barrett’s epithelial cells and nontumorous gastric cardia mucosa. Semiquantitatively, each specimen was placed into one of the following groups: 0% positive cells, 1–10% positive cells, 11–50% positive cells, 51–80% positivity, and 89–100% positivity. The recording of positive tumor cells was based on all of the investigated tumor tissue, estimating the fraction and counting of 5–10 high-power fields. The intensity of the fluorescent reaction product for each was also evaluated each time (0–4+). Only reaction products of 4+ were recorded as positive. With each experiment, a known positive carcinoma control and a negative control were processed. Also, nonspecific reactivity was evaluated by paralleling a section with PBS, replacing primary antibody to each of the oligosaccharide epitopes.

Statistical Analysis. Statistical evaluation of the data was performed using the Student t test. A two-tailed analysis was carried out. Only results with \(P<0.05\) were regarded as significant. These analyses were performed in the Biostatistics Core Laboratory of the University of Colorado Cancer Center.

Results

Le\(^{a}\) expression by tumors arising in Barrett’s epithelium is markedly decreased when compared with adenocarcinomas arising in gastric cardia mucosa (Table 1). Fifty-nine percent of the Barrett-associated adenocarcinomas do not express this epitope, whereas only 18% of non-Barrett adenocarcinomas are negative. Additionally, when Le\(^{a}\) expression was present in the Barrett adenocarcinomas, the percentage of cells expressing the epitope was significantly reduced compared with the non-Barrett cases. All Barrett adenocarcinomas had less than 50% Le\(^{a}\)-positive tumor cells as shown in Fig. 1. Most of the non-Barrett cases contained between 50 and 100% of the tumor cells expressing Le\(^{a}\), as demonstrated in Fig. 2. This difference between Barrett’s and non-Barrett’s cases was statistically significant (\(P<0.001\)).

<table>
<thead>
<tr>
<th>Adenocarcinoma</th>
<th>% Positive cells</th>
<th>Barrett’s adenocarcinoma (%)</th>
<th>Dysplastic epithelium (%)</th>
<th>Intestinal metaplasia (%)</th>
<th>Normal metaplasia (%)</th>
<th>Non-Barrett’s cardia (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>59</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>1–10</td>
<td>18</td>
<td>71</td>
<td>59</td>
<td>18</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>11–50</td>
<td>23</td>
<td>23</td>
<td>35</td>
<td>18</td>
<td>12</td>
<td>30</td>
</tr>
<tr>
<td>51–80</td>
<td>0</td>
<td>6</td>
<td>6</td>
<td>64</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>81–100</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>22</td>
</tr>
</tbody>
</table>

Gastric cardia epithelium in both patient groups expressed similar percentages of Le\(^{a}\), and the areas of junctional type epithelium did not show any clear differences from nontumorous gastric cardia. Table 1 shows the progressive loss of the Le\(^{a}\) epitope by abnormal cells as intestinal metaplasia occurs and progresses to dysplasia in patients with Barrett’s esophageal adenocarcinoma. The decrease of Le\(^{a}\) did not correlate with patient age or with tumor differentiation (\(P>0.5\)). Moreover, we observed no morphological changes between Le\(^{a}\)-expressing cells and cells which were nonexpressors within the same tumor.

The present study also examined expression of Le\(^{a}\) and Le\(^{a}\)-Le\(^{x}\) by normal, metaplastic, dysplastic, and cancer cells of each of the 50 cases. Expression of Le\(^{a}\) and Le\(^{a}\)-Le\(^{x}\) was similar among both subsets of patients (results not shown).
Fig. 1. Less than 50% of the adenocarcinoma cells arising in Barrett's esophagus are Le\textsuperscript{+} positive. A. H&E-stained section of a patient tumor and B. FITC immunofluorescent microscopy of adjacent serial section. \( \times 200 \). Bar. 150 \( \mu \)m.

sensitive compared with the immunoperoxidase technique which is more stable and may be more accurate when considering morphology. The fraction of positive cells was recorded identically with both techniques. Throughout the course of this study, parallel sections were independently evaluated by three different pathologists utilizing both techniques. Notably, no cases negative with the immunoperoxidase technique were scored positive with immunofluorescence and vice versa. Since the pathologists evaluating the different techniques evaluated only one technique or the other, there was no inter/intraobserver variation at this level.

Discussion

A number of anti-Lewis antibodies have been evaluated as probes for cancer markers (10, 20–22). Previously, changes in expression of Lewis antigens have been observed in human intestinal metaplasia, gastric adenomas and gastric carcinomas (9, 23, 24). Human gastric cancers show an enhanced expression of Le\textsuperscript{a} and loss of ABH (17). Distal colon cancers have been shown to express Le\textsuperscript{b} and Le\textsuperscript{a} aberrantly (7). Sialyll-dimeric Le\textsuperscript{a}, an oncodevelopmental carbohydrate antigen, has been shown to be expressed in human colorectal carcinomas, on both glycolipids and mucin proteins, and long and short chain Le\textsuperscript{a} antigens are significantly enhanced in colonic carcinoma (13). It has been postulated that several discrete cell populations at different stages of progression of tumors show variable patterns of glycosylation, and a single tumor can show mosaicism in the expression of carbohydrate antigens (14).

The present study corroborates the findings of others, expands the hypothesis of mosaicism, and confirms changes in epitope expression with epithelial transformation (23, 24). Furthermore, this study suggests possible differences in carbohydrate expression by malignant cells when tumors vary etiologically. Those adenocarcinomas of the distal esophagus which were preceded by intestinal metaplasia and dysplasia and clinically evolved secondary to chronic gastric reflux (Barrett’s) contained subsets of transformed cells which progressively lost their Le\textsuperscript{a} cell surface epitope. Non-Barrett adenocarcinomas arising without documented Barrett’s epithelium and symptoms which suggested the presence of gastric epithelium within the distal esophagus retained the expression of the Le\textsuperscript{a} molecule (Table I). Documentation of the gradual decrease in Le\textsuperscript{a} expression from normal gastric cardia via intestinal metaplasia via dysplasia to invasive adenocarcinoma was possible in the Barrett cases. These changes are not present in the non-Barrett adenocarcinomas studied. In this analysis, it is possible that a Barrett-derived adenocarcinoma was misassigned if the carcinoma obliterated the preexisting metaplasia. Also, it is possible that a carcinoma arising in gastric cardia could invade nearby Barrett's epithelium and be incorrectly assigned. However, any misassignments should tend to blur any real differences in Barrett- and non-Barrett-derived adenocarcinomas. Such error in assignments, if they occur, would not be expected to artificially create differences in marker expression that do not exist. Thus, the uncertainty should not compromise our general results.

Presently, it is unknown whether the decrease in Le\textsuperscript{a} expression occurs early in the evolution of Barrett’s epithelium.

Fig. 2. Non-Barrett’s adenocarcinomas (those not previously associated with reflux) contain tumor cells which are high in the expression of Le\textsuperscript{+}. 80% of these cells are Le\textsuperscript{+} positive. A. H&E-stained section of a patient tumor and B. FITC immunofluorescent microscopy of adjacent serial section. \( \times 200 \). Bar. 150 \( \mu \)m.
Longitudinal studies of patients with Barrett’s esophagus are under way to explore further the evolution of these changes comparing junctional-type Barrett’s epithelium with normal gastric cardia and specialized type to determine whether a decrease occurs in junctional epithelium and progresses through specialized epithelium. This study suggests that Lea expression may be a useful tool in following patients with Barrett’s epithelium, since a decrease of Lea expression by these cells appears to herald the onset of progressive disease.

Acknowledgments
A special thanks to laboratory technician Kirsten Jorgensen for continual dedication to this project, Dr. Iflat Rahim for assistance in manuscript preparation, and Dr. Phil Archer for statistical analysis of the data.

References
Decrease in Le(x) expression in esophageal adenocarcinomas arising in Barrett’s epithelium.

U Engel, R McCombs, P Stranahan, et al.


Access the most recent version of this article at: [http://cebp.aacrjournals.org/content/6/4/245](http://cebp.aacrjournals.org/content/6/4/245)

Sign up to receive free email-alerts related to this article or journal.

To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.