

# Distribution of Integrin Cell Adhesion Molecules in Endometrial Cancer

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***Integrins are ubiquitous cell adhesion molecules that are involved in maintaining normal tissue morphology and have been implicated in the behavior of certain malignancies. We examined the expression of nine integrin subunits in 38 endometrial adenocarcinomas using immunohistochemistry. The pattern of integrin expression in the cancers was compared with that seen in the endometrium of 20 normal cycling women and 7 postmenopausal women. Integrin expression was correlated with grade, stage, nodal status, depth of invasion, steroid receptor status, and histological pattern. In endometrial cancers there was an inverse relationship between the number of integrins expressed and histological grade (P = 0.011). Of the normally expressed, constitutive endometrial epithelial integrin subunits ( $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 6$ , and  $\beta 4$ ), the least frequently seen in the cancers was the  $\alpha 3$  subunit (44.7%) and the most frequently found was  $\alpha 6$  (81.6%). The  $\alpha 5 \beta 1$  integrin, a fibronectin receptor normally found only on endometrial stromal cells, was seen in 17.8% of cases of these epithelial cancers. In addition, a significant association was found between the loss of the  $\alpha 2 \beta 1$  integrin and the presence of lymph node metastases (P < 0.001). These data suggest that a decline in integrin expression occurs more frequently in poorly differentiated endometrial cancers and that the loss of specific integrins may be associated with metastatic nodal spread. (Am J Pathol 1995, 146:717–726)***

Endometrial adenocarcinoma is the most common malignancy of the female reproductive tract. Fortu-

nately, most of these cancers are still confined to the uterus at the time of diagnosis. Although there are fewer deaths associated with endometrial cancer than with other gynecological malignancies, metastatic spread adversely affects prognosis. The ease with which the primary tumor can be evaluated in endometrial cancer offers a unique opportunity to study the differences between metastatic and nonmetastatic disease and to investigate the role of certain cellular characteristics in the biological behavior of these tumors.

One class of cell proteins that may influence cellular phenotype is the integrins, a family of cell surface receptors that recognizes and binds to the extracellular matrix.<sup>1–3</sup> Integrins participate in a wide range of physiological processes, including embryogenesis, wound healing, the immune response, and the behavior of malignant cells.<sup>4</sup> These important proteins mediate the interaction between cells and their environment, direct gene expression, and are thought to play a role in the maintenance of cell shape and tissue architecture.<sup>5–7</sup> Integrins exist within the cell membrane as heterodimeric  $\alpha$  and  $\beta$  subunits and participate in cell-cell and cell-substratum attachment through extracellular ligand binding. At least 14 different  $\alpha$  and 8 different  $\beta$  subunits pair in predictable associations to yield an ever increasing population of integrins.<sup>8</sup>

We recently reported normative data on the pattern of integrin expression in the endometrium of fertile and infertile women<sup>9</sup> and demonstrated that this tissue exhibits complex temporal and spatial changes in integrin expression across the normal menstrual cycle.<sup>10</sup> The purpose of the present study was to investigate the patterns of integrin expression in endometrial epithelial cancers and to determine whether these changes in integrin expression correlate with the clinical behavior of these tumors. As integrins are

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important for the maintenance of normal tissue morphology, we reasoned that alterations in the phenotypic appearance of endometrial tumors and their invasive potential may relate directly to the expression of these specific cell adhesion molecules. Biochemical markers of cell differentiation that predict biological behavior may have significant prognostic value and help to direct therapy in endometrial cancer.

## **Material and Methods**

### **Endometrial Samples**

Tissue samples from 40 women were snap-frozen at the time of laparotomy for primary ( $n = 36$ ) or recurrent ( $n = 4$ ) endometrial adenocarcinoma at Duke University Medical Center. The patients ranged in age from 26 to 80 (mean age 63.8) years. There were 10 well differentiated, 20 moderately differentiated, and 10 poorly differentiated cancers. There were 17 stage I/II tumors, 18 III/IV tumors, and 4 recurrent tumors. Histological types included endometrioid ( $n = 31$ ), adenosquamous ( $n = 5$ ), and papillary serous ( $n = 4$ ). In 24 cases, lymph node dissection was performed. In 36 samples, estrogen receptor content was determined and 29 were evaluated for progesterone receptors. Depth of invasion was recorded for all primary tumors. Samples of normal endometrium were obtained from 20 normal fertile women of reproductive age and 7 menopausal women at the time of hysterectomy for benign disease. Histological dating was available for all samples from cycling individuals and was in agreement with the chronological dating based the basis of the last menstrual period. Endometrium from fertile controls was obtained in the luteal phase, when integrin expression is known to be maximal.<sup>10</sup>

### **Antibodies**

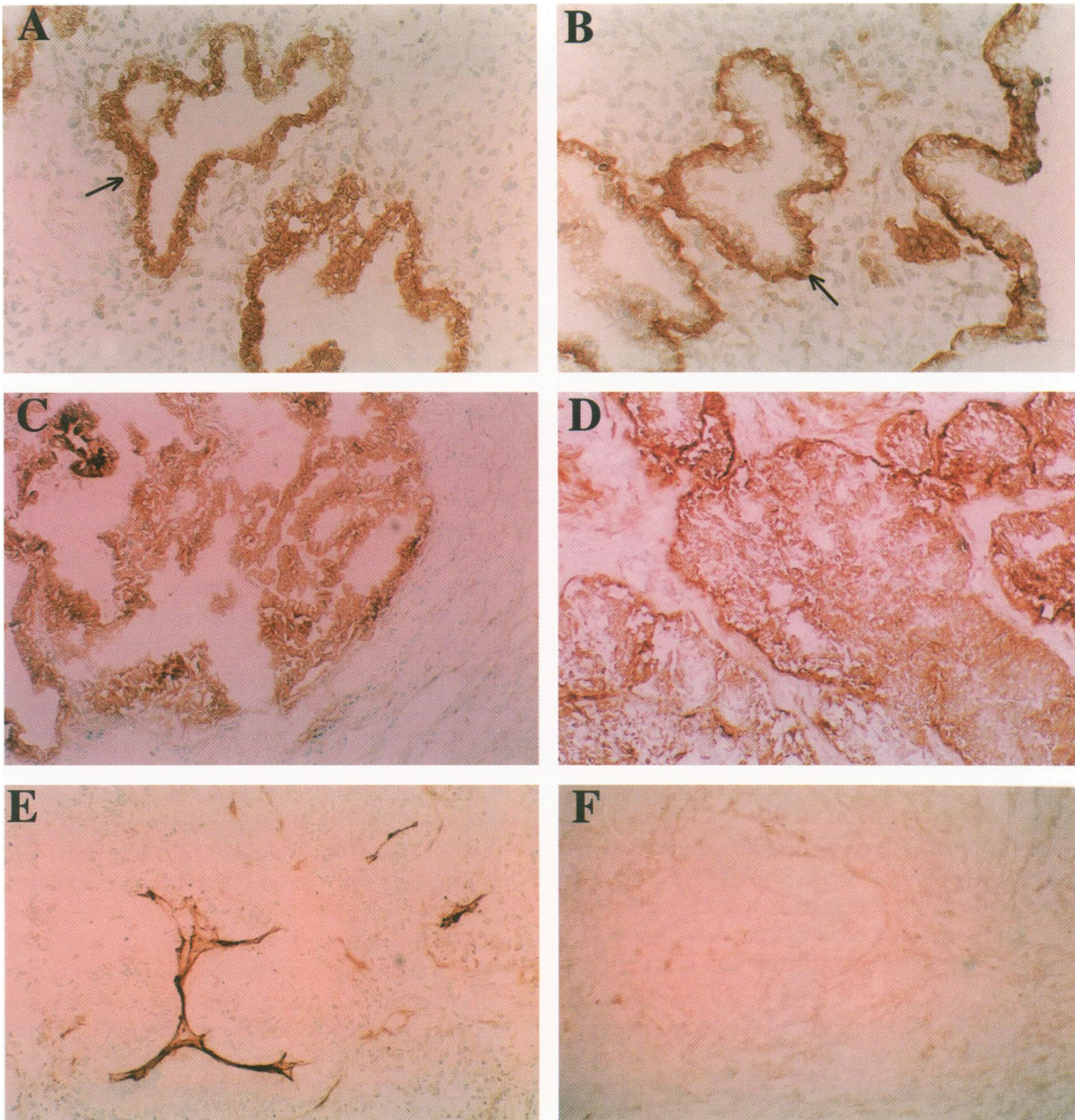
Monoclonal antibodies (MAbs) P1H5, P1B5, P1D6 specific to the  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 5$  subunits, respectively, were generously provided by Drs. Elizabeth Wayner and William Carter. MAbs TS2/7 directed against the  $\alpha 1$  subunit and B5H10 directed against the  $\alpha 4$  subunit were a gift from Dr. Martin Hemler. GoH3, a specific MAb directed against  $\alpha 6$ , was donated by Dr. Arnoud Sonnenberg. Drs. Joel Bennett and James Hoxie provided MAb SSA6 specific to the  $\beta 3$  subunit and MAb LM142 against  $\alpha v$  was provided by Dr. David Cheresh. The  $\beta 4$  antibody was generously sup-

plied by Dr. Steven Kennel. Each of these antibodies has been previously characterized.<sup>11-16</sup>

### **Immunohistochemistry**

Immunoperoxidase staining was performed on cryostat sections of normal and malignant endometria. Serial cryosections, 8  $\mu$  thick, were placed onto poly-L-lysine-coated slides, fixed in  $-20$  C acetone for 10 minutes, and stained by using Vectastain Elite ABC kits (Vector Laboratories, Burlingame, CA). Diaminobenzidine (Sigma Chemical Co., St. Louis, MO.) was used as the chromogen. Primary antibody was placed on cryosections after blocking with 1% bovine serum albumin in phosphate-buffered saline (PBS) and allowed to bind at room temperature for 1 hour. A PBS, pH 7.2 to 7.4, rinse was followed by secondary antibody consisting of biotinylated goat anti-mouse antibody for 30 minutes. After a PBS rinse, endogenous peroxidases were quenched by a 30-minute incubation with 0.3%  $H_2O_2$  in absolute ethanol, followed by a 30-minute rehydration in PBS. Sections were incubated in avidin-biotinylated horseradish peroxidase macromolecular complex before addition of diaminobenzidine for 3 minutes to complete the reaction. Samples were subsequently washed in PBS and mounted.

The resulting staining was evaluated by two observers in a blinded fashion on a double-headed microscope at low ( $\times 100$ ) and higher ( $\times 400$ ) magnification. The consensus HSCORE was calculated by the following equation:  $HSCORE = \sum P_i (i + 1)$ , where  $i = 1, 2, \text{ or } 3$  (indicating weak, moderate, or strong staining) and  $P_i$  is the percentage of stained epithelial cells for each intensity, varying from 0 to 100%. Low intraobserver ( $r = 0.983$ ;  $P = 0.00001$ ) and interobserver differences for HSCORE ( $r = 0.994$ ;  $P = 0.00001$ ) in a large scale study of uterine adenocarcinoma have been previously reported for this technique.<sup>17</sup> An HSCORE of 0.5 or less was not clearly distinguishable from no staining and used as the threshold value of a negative result. Examples of variations in HSCORE are presented in Figure 1 (see Results). Statistical analysis was performed by using SAS (SAS Institute, Cary, NC). Multiple logistic regression was performed to evaluate the association between integrin expression (HSCORE) and metastatic spread after adjusting for the potential confounding effects of age, stage, grade, histology, and receptor status. Associations between variables were examined by using the Mantel-Haenszel Spearman correlation test. Due to the small sample size,  $P$  values



**Figure 1.** Photomicrographs showing immunostaining for  $\alpha 2$  (A, C, and E) and  $\alpha 6$  (B, D, and F) integrin subunits in normal endometrium (A and B,) and endometrial cancer (C to F). Note the positive expression of each integrin in normal and malignant samples (A to D) compared with the loss of expression in other cases (E and F). In cases with  $\alpha 2$  or  $\alpha 6$  expression (C and D,) staining intensity was reduced or qualitatively different than that for normal glandular endometrium. As cells moved away from the BM (D; asterisks) staining for  $\alpha 6$  becomes more diffuse and no longer spatially localized to the basal portion of the cells. C, stage IB, grade I; D, stage IIIA, grade III; E, stage IIIC, grade II; F, stage IB, grade III. Magnification,  $\times 200$ .

were also obtained by using Fisher's exact test (two tail). Photomicrographs were made with Kodak Ektar 100 ASA film.

### Results

Among 40 samples of adenocarcinoma of the endometrium, 38 were suitable for immunostaining with

identifiable tumor present. Expression of constitutive integrins ( $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 6$ , and  $\beta 4$ ) was uniformly present in all normal endometria. Expression of the cycle-specific integrins of  $\alpha 1$ ,  $\alpha 4$ , and  $\beta 3$  was identified only in the samples from cycling women and absent from all menopausal samples. Integrin expression varied in the endometrial cancers. Results are summarized in Table 1, which includes the ligand preference of



**Table 1.** *Distribution of Integrins in Normal and Malignant Endometrium*

Integrin subunit	Ligand preference	Integrin expression (%)		
		Normal cycling	Normal menopausal	Malignant
$\alpha 1$	C/LM	20/20 (100)	0/7 (0)	5/38 (13.2)
$\alpha 2$	C/LM	20/20 (100)	7/7 (100)	26/38 (68.4)
$\alpha 3$	C/LM/FN	20/20 (100)	7/7 (100)	17/38 (44.7)
$\alpha 4$	FN	20/20 (100)	0/7 (0)	1/38 (2.6)
$\alpha 5$	FN	0/20 (0)	0/7 (0)	5/38 (13.1)
$\alpha 6$	LM	20/20 (100)	7/7 (100)	31/38 (81.6)
$\alpha v$	FN <sup>†</sup>	20/20 (100)	7/7 (100)	30/37 <sup>*</sup> (81.8)
$\beta 3$ ( $\alpha v\beta 3$ )	FN/VN/OP/BSP/LM/TBSN/vWF	20/20 (100)	0/7 (0)	10/38 (26.3)
$\beta 4$ ( $\alpha 6\beta 4$ )	LM	20/20 (100)	7/7 (100)	23/38 (61)

C, collagens; LM, laminin; FN, fibronectin; VN, vitronectin; OP, osteopontin; BSP-1, bone sialoprotein-1; TBSN, thrombospondin; vWF, von Willebrand factor.

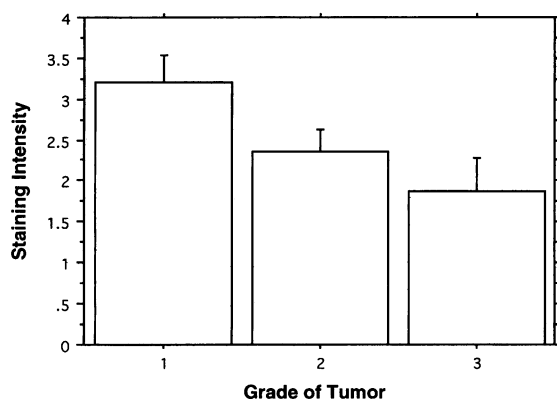
\*Cycling, luteal phase samples.

<sup>†</sup>Ligand preference is dependent on which  $\beta$  subunit  $\alpha v$  is paired with.

\*Reflects missing value.

each integrin. The integrin subunit least frequently expressed in endometrial cancers was  $\alpha 3$ , a promiscuous integrin that binds collagen, laminin, and fibronectin, identified in only 17 of 38 (44.7%) tissues. The  $\beta 4$  subunit, an epithelial integrin subunit that associates exclusively with  $\alpha 6$  to form a laminin receptor,<sup>18</sup> was present in 23 of 38 tumors (61%). The  $\alpha 2$  subunit was detected in 26 samples (68.4%);  $\alpha 6$  (the heterodimer pair to  $\beta 4$ ) was most frequently expressed and found in 31 of 38 tumors (81.6%). Examples of positive and negative staining for  $\alpha 2$  and  $\alpha 6$  in normal and malignant samples are shown in Figure 1.

The relationship between integrin expression and histological grade is shown in Figure 2. We observed a stepwise loss of integrins as tumor grade increased. The mean number of constitutively expressed integrins ( $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 6$ , and  $\beta 4$ ; maximum of four) de-



**Figure 2.** *Loss of integrin expression with increasing grade in endometrial cancer. Four integrins are constitutively expressed on normal endometrial epithelium ( $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 6$ , and  $\beta 4$ ). The mean number of these integrins that was expressed in the various samples was calculated for each grade. Note that, on average, fewer of these four integrins were expressed with increasing grade, on the basis of immunohistochemical staining. Grade was significantly and inversely associated with the mean number of integrins expressed ( $P = 0.011$ ) by Mantel-Haenszel Spearman correlation.*

creased as cellular morphology became increasingly disordered ( $P = 0.011$ ; Mantel-Haenszel Spearman correlation). Among the four, expression of the  $\beta 4$  subunit correlated best and was inversely related to tumor grade ( $P = 0.013$ ). Integrin expression in specimens of recurrent adenocarcinoma (all grade II) was not different from that in primary tumors of similar grade. The distribution and staining intensity (HSCORE) of the four epithelial integrin subunits at each grade was compared with that in 20 normal endometrial tissue specimens. Figure 3 illustrates the trend toward greater heterogeneity and a progressive loss of integrins with increasing tumor grade.

Examination of tissue sections containing both normal and malignant elements allowed direct comparison of the patterns of integrin expression. As shown in Figure 4, cytokeratin (A),  $\alpha 3$  (C),  $\alpha v$  (D),  $\beta 3$  (F),  $\alpha 6$  (G), and  $\beta 4$  (H) were all more highly expressed in normal luminal and glandular epithelium than in the nearby cancer cells (asterisk) in this grade II endometrial cancer. The  $\alpha 1$  subunit was not expressed in normal luminal or glandular epithelium, nor in neoplastic epithelium in this specimen (Figure 4B), and  $\alpha 5$  expression was identified only in the stromal compartment (Figure 4E). Another intriguing observation was that  $\alpha 6$  and  $\beta 4$  (Figure 4G, H), which together form a laminin receptor, were confined to tumor cells in contact with the basement membrane (BM), but not seen on cells that piled up and moved away from the BM. The morphological appearance of this integrin in normal *versus* tumor elements was qualitatively distinct as well, with weaker staining observed on the abnormal glandular elements (see  $\beta 4$  in Figure 4H, asterisk).

Cycle-dependent endometrial integrins ( $\alpha 1$ ,  $\alpha 4$ , and  $\alpha v\beta 3$ ), all present in the midluteal phase of normal cycling endometrium, were frequently absent in tumors and also not expressed in post-menopausal

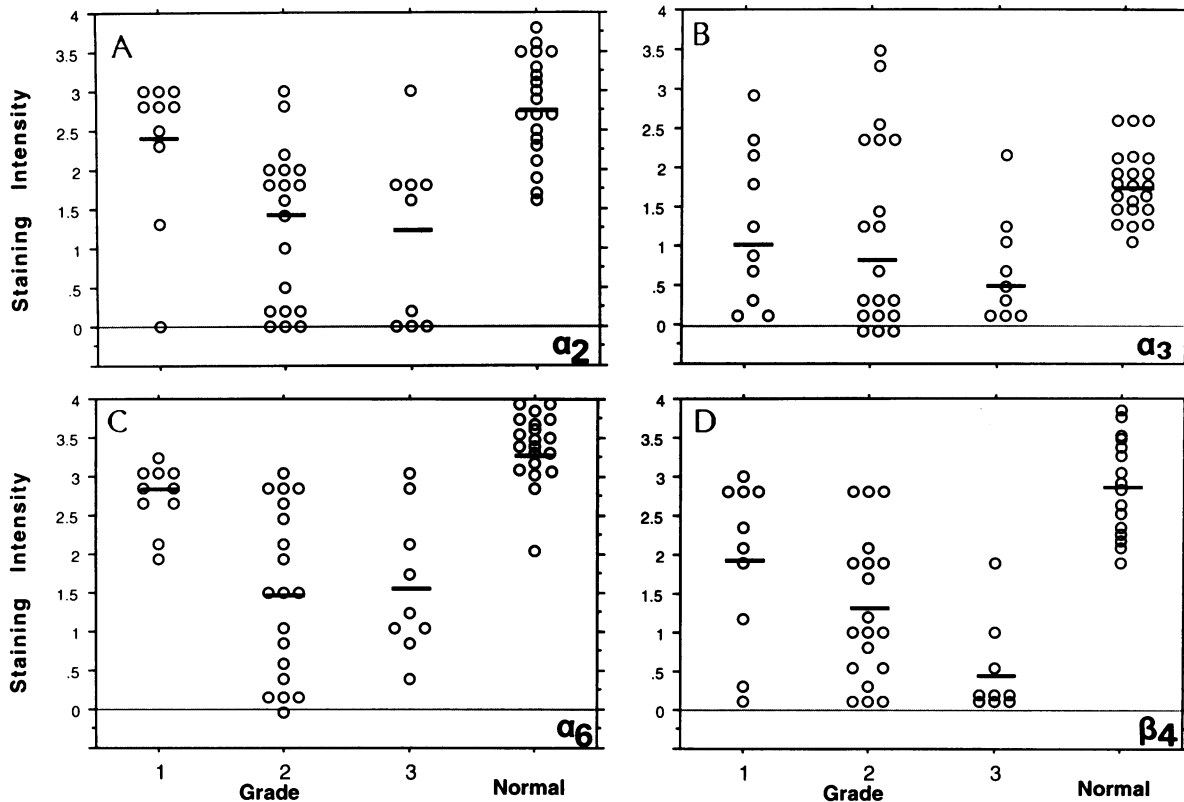


Figure 3. The relative staining intensity of four integrin subunits,  $\alpha_2$  (A),  $\alpha_3$  (B),  $\alpha_6$  (C), and  $\beta_4$  (D) in tumors of various grades (I, II, or III) compared with normal, cycling endometrium.

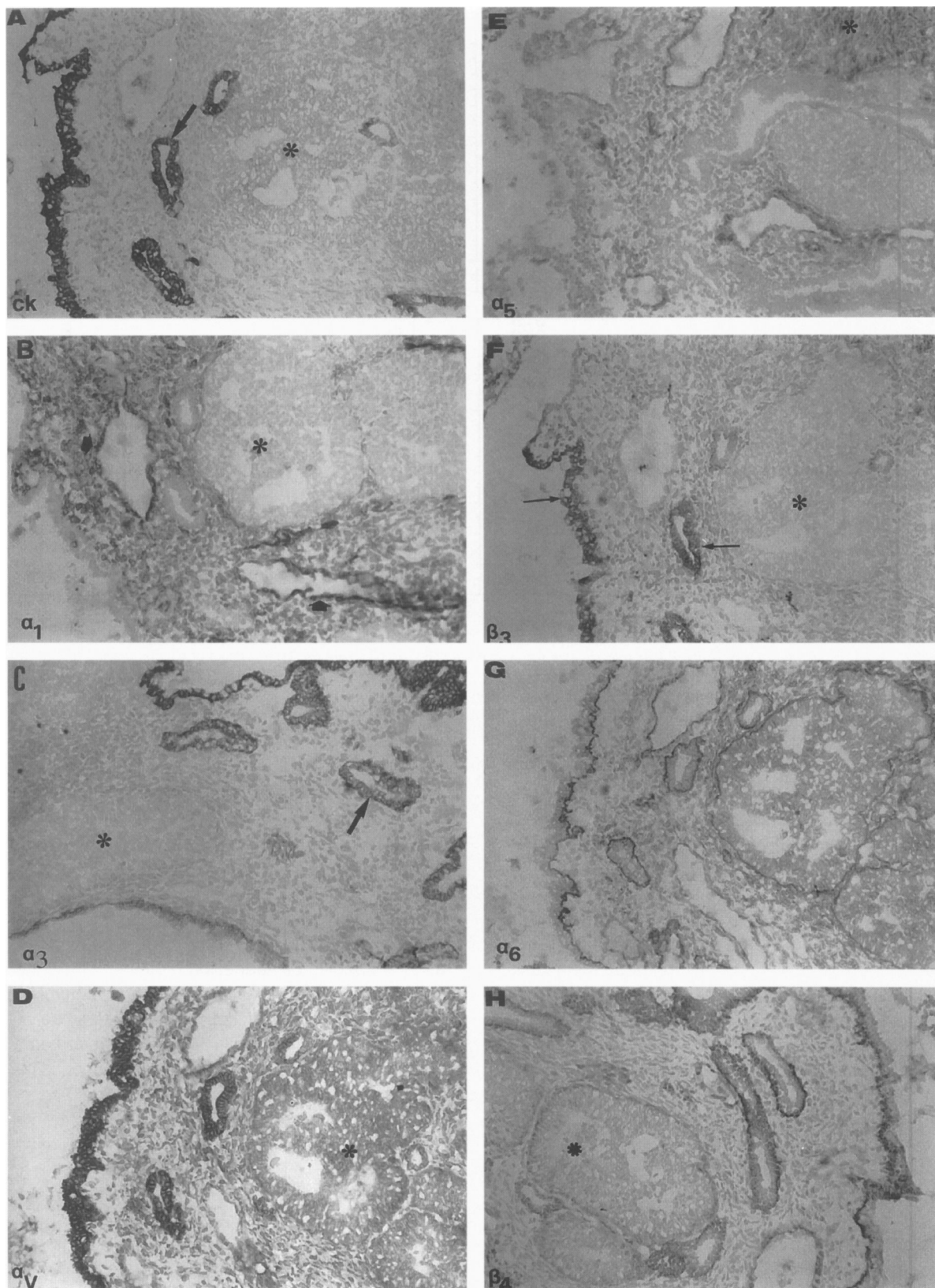
endometrium. Among the 38 adenocarcinoma specimens examined in our study,  $\alpha_1$  was present in only 5 (13.2%) and  $\alpha_4$  was identified in just 1 instance (2.6%). Whereas the  $\beta_3$  subunit was observed in 10 tissue specimens (26.3%), its pairing subunit,  $\alpha_v$ , was frequently expressed in these tumors (85%).

Multiple logistic regression analysis was performed to detect any associations between integrin expression and other parameters such as age, stage, grade, myometrial invasion, lymph node metastasis, depth of invasion, histological type, and steroid receptor status. We found no correlations between histological type or depth of invasion and expression of the four constitutive integrin subunits ( $\alpha_2$ ,  $\alpha_3$ ,  $\alpha_6$ , or  $\beta_4$ ). Furthermore, steroid receptor status and stage did not correlate with the overall number of integrins expressed. However, we found a highly significant association between the loss of  $\alpha_2$  integrin expression and lymph node metastases. In the 24 cases in which lymph node dissection was performed, positive nodes were never observed when the primary tumor expressed  $\alpha_2$  ( $n = 11$ ). Conversely, lymph node metastases were found in 9 of 13 cases (69%) in which  $\alpha_2$  was absent in the primary tumor ( $P < 0.001$ ). The presence or absence of  $\alpha_2$  was equally distributed by

grade. Aberrant  $\alpha_2$  expression was associated only with lymph node status and did not correlate, either positively or negatively, with grade, local spread to surrounding structures, or depth of tumor invasion.

### Discussion

Epithelial cells maintain a polarized phenotype, adhering to the BM and adjacent cells through elaboration of specific cell-adhesion proteins.<sup>19</sup> Endometrium maintains many of the same integrins commonly expressed on other epithelia.<sup>2,19</sup> In this study, we examined the integrin profile in 38 cases of endometrial adenocarcinoma in comparison with that seen in the endometrium of 20 normally cycling and 7 postmenopausal women. Significant differences in integrin expression were noted between normal and malignant cells. Although an altered pattern of integrin expression has been demonstrated in a wide variety of cancers,<sup>20-28</sup> this represents the first broad survey of integrins in endometrial cancer. Our findings suggest that integrin expression is related to the degree of differentiation of the tumor and that the loss of a specific integrin ( $\alpha_2\beta_1$ ) may predict or predispose to lymph node metastasis in this disease.



**Figure 4.** Photomicrograph of immunohistochemical staining of a stage IB (grade II) endometrial cancer found adjacent to normal glandular and luminal epithelium. Cytokeratin staining (ck; A) was lacking in the malignant element (asterisk) relative to the normal glandular cells (arrow). Reduced immunostaining was also noted for  $\alpha_3$  (C),  $\alpha_v$  (D),  $\beta_3$  (F), and  $\alpha_6\beta_4$  (G and H). Note the loss of polarity of staining for the latter two subunits ( $\alpha_6$  and  $\beta_4$ ) as the cells move away from the BM (G and H). Magnification,  $\times 200$ .

In general, human cancers tend to express the same integrins as the cells from which they originate.<sup>29</sup> The appearance of new, uncharacteristic integrins<sup>21,30-33</sup> and the loss of appropriate integrin expression<sup>24,26,28,34-37</sup> have previously been described. For example,  $\alpha 1$  can be found in high stage bladder cancer but is not in low stage cancers and normal bladder epithelium.<sup>34</sup> The  $\alpha 2$  subunit may be lost in breast cancer tissue<sup>24,26</sup> or increased in certain prostate cancers.<sup>31</sup> In human endometrial epithelium, constitutive expression of  $\alpha 2$ ,  $\alpha 3$ ,  $\alpha 6$ , and  $\beta 4$  has previously been described in normal tissues.<sup>9,38</sup> The majority of cancers surveyed in our study expressed one or more of these same integrin subunits. The appearance of integrins not normally present in native endometrial epithelium (e.g., the  $\alpha 5\beta 1$  fibronectin receptor) was uncommon and was not associated with invasiveness or metastases.

One barrier to a better understanding of the mechanisms controlling the spread of cancer cells is the complexity of the metastatic process itself. The cascade of events leading to metastasis likely involves aberrant adhesion characteristics in the transformed cells.<sup>39</sup> Cells must first grow in number, detach from existing extracellular matrix, invade through BM, enter vascular or lymphatic channels to travel to distant sites and then reassociate within the new tissue matrix. A reduced or lost ability to make and/or bind extracellular matrix components<sup>40</sup> and the elaboration of specific extracellular matrix digesting enzymes<sup>41,42</sup> may also contribute to this process. Modification of the invasive phenotype by signal transduction through extracellular matrix binding to integrins may cause an arrest of motility<sup>43</sup> or contribute to the spread to distant sites.<sup>7</sup> In general, there is a tendency for malignant epithelial cells to lose certain integrins,  $\alpha 2$ ,  $\alpha 3$ , and  $\alpha 6$ , for example, as the tumors become less differentiated and morphologically more disordered.<sup>20,24,25,28,36,37,44,45</sup>

Previous studies have also documented increased integrin expression in certain tumors<sup>22,30,31,33,46,47</sup> and provided evidence that altered patterns of integrin expression may influence the biological behavior of the tumor. For example, the expression of  $\alpha v\beta 3$  in melanoma cells has been associated with increased invasiveness,<sup>21</sup> whereas expression of  $\alpha 4$  decreases the invasive phenotype of melanoma cells,<sup>48</sup> and overexpression of  $\alpha 5\beta 1$  moderates the transformed phenotype in Chinese hamster ovary cell.<sup>49</sup> The most significant finding in our study on human endometrial cancer was the striking association between absent  $\alpha 2$  expression in the primary tumor and lymph node metastases. Zutter et al<sup>24</sup> observed reduced  $\alpha 2$  expression in poorly differentiated adenocarcinoma of

the breast, although the finding did not correlate with lymph node involvement. Koretz et al<sup>26</sup> found that reduced  $\alpha 2$  expression was associated with advanced stage of colon cancers and that metastases tended to exhibit lower levels of  $\alpha 2$  expression than the primary tumor. In contrast, Bonkhoff et al<sup>31</sup> observed increased  $\alpha 2$  (and  $\alpha 6$ ) expression in metastatic cells as compared with those in primary prostate cancer. Chan and co-workers<sup>50</sup> have shown that rhabdomyosarcoma cells transfected with the  $\alpha 2$  subunit (which do not normally express this integrin) were more metastatic when injected into nude mice, but because intravascular injection of malignant cells bypasses the early steps in the natural spread of primary tumors, their observations must be interpreted with caution.

Cancer cells appear to undergo changes in integrin expression that parallel the loss of normal tissue architecture and cellular behavior. Alterations in the appearance of BM receptors such as  $\alpha 6$  and  $\beta 4$  are often seen in malignant epithelial tumors.<sup>24,28,51</sup> In endometrial cancers, as tumor grade increases, the piling up of endometrial epithelium is associated with altered  $\alpha 6\beta 4$  integrin topography. Specifically, only those cells that move away from the BM fail to express either subunit. The lack of BM has previously been shown to be associated with reduced  $\alpha 6\beta 4$  expression in human mammary carcinoma,<sup>51</sup> and a redistribution of BM receptor subunits ( $\alpha 2$  and  $\alpha 6$ ) has been described in prostate cancer cells.<sup>32</sup> However, whether these changes in tissue morphology precede the loss of integrins or result from the lack of BM is not yet clear. Basement membrane contact may in some way induce the appearance of these integrins on the basal pole of epithelial cells as previously suggested.<sup>51</sup> The concomitant loss of both  $\alpha 6$  and  $\beta 4$ , as shown in this and in other studies, supports this hypothesis.

In normal endometrial epithelium, the expression of several integrins is clearly cycle dependent.<sup>9</sup> Expression of both the  $\alpha 1$  and  $\alpha 4$  integrin subunit appears to be progesterone dependent, first identified on endometrial glands at the time of ovulation.<sup>10,38</sup> The vitronectin receptor  $\alpha v\beta 3$  appears highly regulated as well, expressed only during the mid- and late luteal phase.<sup>9,10</sup> The infrequent expression of these integrins in both menopausal and transformed endometrium should, therefore, not be surprising. As shown in Figure 4, certain tumors expressed  $\beta 3$  but not  $\alpha 1$ , a situation not usually seen in cycling endometrium. Because growth factors have been shown to regulate the expression of the  $\alpha v\beta 3$  integrin in several cell types,<sup>52-58</sup> it is possible that abnormal regulation of growth factor expression may account for this unex-

pected pattern of integrin expression. Such observations may help identify the factors that regulate the  $\alpha v \beta 3$  vitronectin receptor in normal human endometrium or explain its loss in certain infertility states.<sup>59</sup> The higher incidence of  $\alpha v$  in tumors (85%) compared with  $\beta 3$  (26%) is consistent with the observation that  $\alpha v$  may pair with many different  $\beta$  subunits.<sup>8</sup>

In summary, the spectrum of integrin expression in cancer of the endometrium parallels that in normal endometrial epithelium. A general loss of integrin expression was observed as tumor grade increased with loss of  $\alpha 6 \beta 4$  the most highly correlated with increasing grade. Absent  $\alpha 2 \beta 1$  integrin expression in the primary tumor was highly associated with lymph node metastases, independent of the stage or depth of invasion, an observation that may have clinical applications. Given the accessibility of the primary tumor in this form of cancer, integrin status could potentially help direct therapy or predict prognosis.

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