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Large-Cell Acanthoma of the Skin

A Study by Image Analysis Cytometry and Immunohistochemistry

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Although large-cell acanthoma is a well-known clinicopathological entity, its biologic spectrum and nature are still subject to debate. We studied seven cases of large-cell acanthoma by image analysis cytometry for DNA content and by immunohistochemistry, using antibodies to proliferating cell nuclear antigen (PCNA)/cyclin. The data were compared with individual cases of seborrheic keratosis (SK), actinic keratosis (AK), and Bowen's disease (BD). The DNA distribution of large-cell acanthoma was variable. There were varying peaks at the DNA index values of 1 and 2 (diploid and tetraploid values), but all cases contained a significant aneuploid population between DNA index of 1 and 2. The mean DNA index was 1.44 (1.27–1.77); 1–20% of the cells exceeded 2, and 0–2% exceeded 3. The DNA index for lesions in the other differential diagnostic groups studied was as follows: SK, 1.0; AK, 1.4; BD, 1.8. The percentage of cells with positive nuclear staining for PCNA/cyclin was <20% in all cases of large-cell acanthoma. The discrepancy between the high number of aneuploid and tetraploid cells observed on the DNA distribution curve and the lack of evidence for significant proliferation based on immunohistochemical stains suggest that these cells are resting cells with abnormal DNA clone. Although these results provide additional information about the biologic nature of large-cell acanthoma, they do not resolve the controversial nosologic status of lesions in this histologic group.
Key Words: Large cell acanthoma—Image analysis cytometry—Proliferating cell nuclear antigen.

Large-cell acanthoma (LCA), an entity first identified by Fand and Pinkus in 1970, comprises a spectrum of lesions (1,2). LCA was defined as a plaque occurring on sun-exposed skin with the histologic hallmark of large, sharply demarcated, relatively uniform keratinocytes that exhibit hypergranulotic-orthokeratotic maturation (3). Recently, several investigators attempted to resolve the controversial nosologic aspects of this lesion based on clinical, histologic, and—to some extent—DNA ploidy studies (4–11). Although there has been general agreement that the typical lesion is composed of large cells, or at least cells with enlarged nuclei, there is a wide range of speculation regarding the interpretation of the frequently associated changes—i.e., elongation of the rete ridges, basal layer hyperpigmentation, cytologic atypia, etc.—that are sometimes observed in these cases (8–11). In short, the controversy has centered around the question of whether LCA should be considered a distinct diagnostic group, or an unusual variant of keratosis, including those types that may have potential for biologic progression.

While we are aware of the limitations of the DNA ploidy studies, since some of the earlier arguments were based on the DNA content of these lesions (1,9,10), we intended our study to provide additional data on LCA by using DNA cytometry in correlation with an immunohistochemical marker of proliferation.

MATERIALS AND METHODS

Seven cases originally diagnosed as LCA were retrieved from the files of the Department of Pathology, University of Iowa, Iowa City, IA, U.S.A.;

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and the Department of Pathology, St. John's Mercy Medical Center, St. Louis, MO, U.S.A. The glass slides were reviewed to confirm the diagnosis of LCA. Also, individual cases of seborrheic keratosis (SK), Bowen's disease (BD), and actinic keratosis (AK) were selected for comparison of DNA ploidy results.

DNA content analysis was performed as reported earlier (12). Seven-micron sections cut from the paraffin blocks were stained with hematoxylin and eosin (H&E) to select the area for analysis, then with modified Spectra Feulgen stain (12–14) for DNA content analysis. Sections were viewed with a Zeiss microscope using a ×40 lens without oil or a substage condenser. Uniformly stained representative fields were carefully selected for the study (15). A Hamamatsu video camera transferred the images to a Gould monitor, where the images were digitized into a computer memory on 512 × 512 pixel arrays (16–18). The Gould IP8500 and Silicon Graphics image processing systems were used to trace individual nuclei. Approximately 100 keratinocytes and 100 lymphocytes were traced in each section.

The data were then transferred to a Macintosh II computer and analyzed with the Excel software. DNA content was standardized by multiplying the area (in square pixels) and optical density for each nucleus. Due to the different staining intensity of the individual slides, the normal diploid DNA content was determined individually for every case. Histograms of the lymphocytes were used to determine the normal diploid content (2N). Histograms were obtained by plotting DNA content against frequency of representation. The DNA content was expressed as DNA index (DNA content/mean DNA content of normal diploid cells). By definition, the DNA index of normal cells is 1. Exceeding rates for DNA index of 2, 2.5, and 3 were calculated and expressed as a percent of all nuclei counted.

For the immunohistochemical studies, formalin-fixed, paraffin-embedded tissue was used. Sections were deparaffinized in xylene, rehydrated in graded alcohols, and rinsed. Endogenous peroxidase activity was blocked using 0.3% H_2O_2/distilled water. Anti-proliferating cell nuclear antigen (PCNA) (PC 10 clone, Signet, Dedham, MA, U.S.A.) at a dilution of 1:35 was applied for 1.5 h at room temperature. The signal was detected with a standard avidin-biotin complex using 3,3 diaminobenzidine as the chromogen with hematoxylin as a counterstain.

Germinal center staining in a lymph node was used as an external positive control. Staining of the basal keratinocytes of the uninvolved epidermis was assessed as an internal control in each case. Negative controls consisted of tissue sections with substitution of mouse serum for the primary antibody.

The individual cases were evaluated for staining of the keratinocytes of the epidermis; we recorded the percentage of cells that showed reactivity and the distribution of reactivity. In each case, multiple high-power fields (×400) were evaluated.

RESULTS

Light Microscopy

The seven cases of LCA had features similar to those already described and illustrated by different investigators. These included a keratinocyte population with large, plump nuclei mainly in the lower half of the epidermis associated with variable degrees of basal layer hyperpigmentation, elongation, and bulbous enlargement of rete ridges and orthokeratosis (Fig. 1A). Additional and less often observed changes were a flattened or verrucous appearance of the epidermis, cytologic atypia, necrotic keratinocytes, solar elastosis, and lymphohistiocytic infiltrate in the papillary dermis. Although we observed some of the patterns described by Sanchez Yus et al. (11)—i.e., basic, verrucous, and flat types—we did not subclassify our cases into these groups because of the limited number of cases, incomplete sampling, and the presence of different patterns within the same lesion.

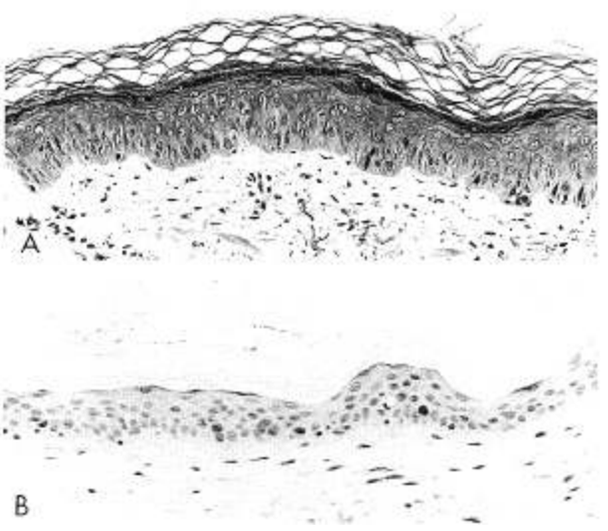

FIG. 1. (A) Light-microscopic view of a large-cell acanthoma. The keratinocytes have enlarged, relatively uniform nuclei. **(B)** Immunohistochemical stain for proliferating cell nuclear antigen (PCNA). Note the low percentage of nuclear labeling.

Image Analysis Cytometry

Frequency distribution of the DNA content for the seven cases of LCA failed to show a consistent pattern. Two cases (cases 3 and 7) had a large peak at 1 (normal diploid value), representing ~50% of the cells. There was no significant population at 2 (tetraploid value) (<10% of the cells). The remainder of the cells were located between the values of 1 and 2 (aneuploid cells) (Fig. 2A). Another pattern (cases 4 and 5) was characterized by a slightly smaller peak at 1 (40% of cells), a small but distinct peak at 2 (~15% of cells), and a large population in between 1 and 2 (Fig. 2B). Two of the seven cases (cases 2 and 6) produced a prominent peak at 2 (≥40% of cells) and a smaller peak at 1 (Fig. 2C); whereas one case (case 1) showed the most irregular pattern, with small peaks at 1 and 2 (20%) and a prominent population above 2 (Fig. 2D). The only feature common to all these cases was a consistent aneuploid population between 1 and 2.

The mean DNA index for all cases of LCA was 1.44 (range, 1.27–1.77). The values for individual cases and the exceeding rate for 2, 2.5, and 3 (corresponding to 2N, 5N, and 6N values) are summarized in Table 1. Case 1, which showed the most irregular DNA distribution, had the highest exceeding rates for 2.5 and 3. Four of the seven cases had no cells above 3.

The DNA distribution of the individual cases of SK, AK, and BD is shown in Fig. 3. The case of SK (Fig. 3A) showed a single peak at 1, consistent with a normal diploid cell population. In contrast, AK (Fig. 3B) had an abnormal pattern with peaks at 1 and 2, but it also had many aneuploid cells in between 1, 2 and 3 and a few cells exceeding 3. The case of BD (Fig. 3C) produced the most irregular and widest frequency distribution, with no obvious peaks at 1 or 2 and most cells having aneuploid values. The DNA index was 1 for SK, 1.44 for AK, and 1.84 for BD.

TABLE 1. *DNA content and DNA distribution*

Case	Mean DNA index	Exceeding rates for DNA index = 2	DNA index = 2.5	DNA index = 3
Large-cell acanthoma				
Case 1	1.77	28	11	5
Case 2	1.69	27	5	2
Case 3	1.27	1	0	0
Case 4	1.27	3	1	0
Case 5	1.38	3	1	0
Case 6	1.56	17	2	0
Case 7	1.18	7	2	1
Seborrheic keratosis	0.95	0	0	0
Bowen's disease	1.84	38	10	4
Actinic keratosis	1.44	11	4	1

Immunohistochemistry

In all seven cases of LCA, the percentage of cells with positive nuclear staining was <20% (Fig. 1B). The positive cells were located predominantly in the basal and suprabasal layers. In one case, there

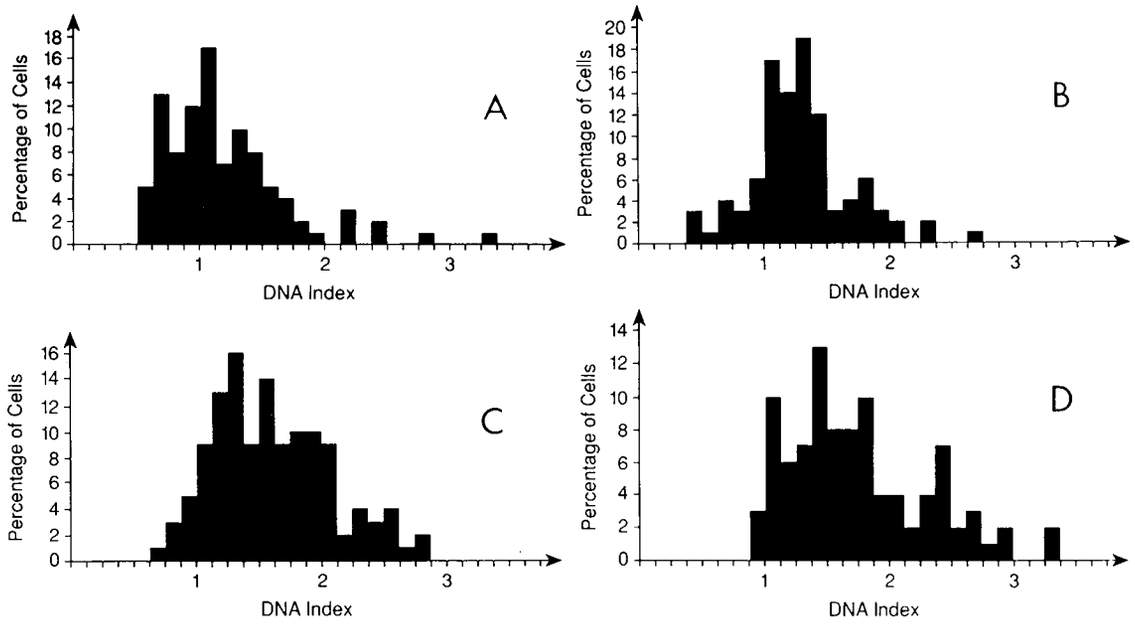

FIG. 2. (A–D) Representative examples of DNA distribution of large-cell acanthoma. The distribution can vary from one having the majority of cells centered around 1 **(A)** to one showing a distinctly aneuploid pattern **(C).**

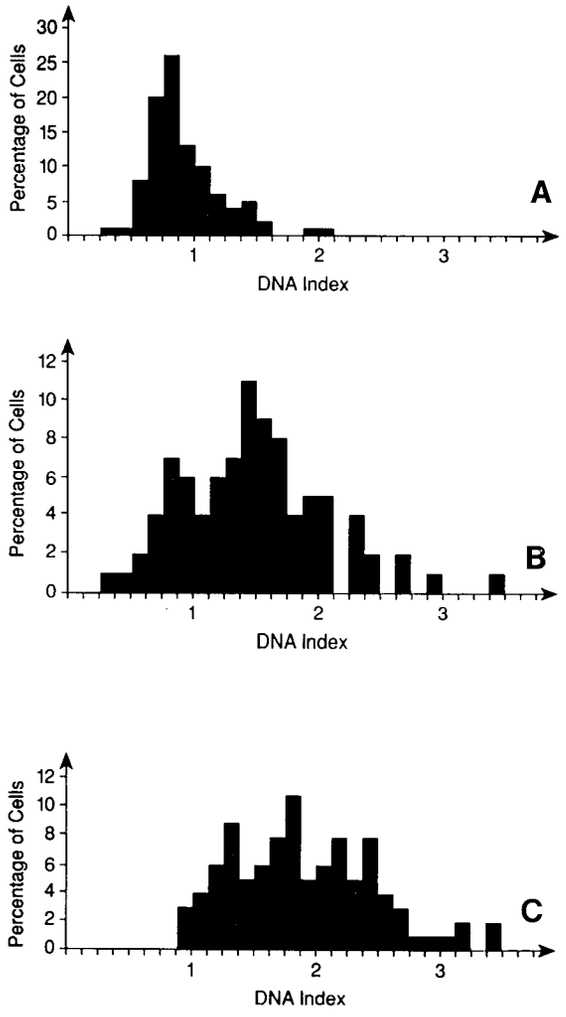

FIG. 3. DNA distribution of representative cases of seborrheic keratosis **(A)**, actinic keratosis **(B)**, and Bowen's disease **(C)** showing the shift of the pattern from the normal diploid population of seborrheic keratosis to the irregular aneuploid distribution of Bowen's disease.

were scattered positive cells in the spinous layer. The adjacent normal-appearing epidermis showed only scant positivity in the basal layer, when histologically normal-appearing skin was present in the specimen. The insufficient amount of normal skin precluded reliable quantification.

DISCUSSION

Currently, there are several theories regarding the classification of LCA. One theory suggests that LCA is a distinct lesion and that it can be differentiated from seborrheic keratosis, solar lentigo, and lichenoid keratosis based on the clinical, histologic, and, in part, on DNA spectrophotometric results (9,10). An alternative theory argues that LCA is not a distinct entity but a variant of solar lentigo and that the lesion is in the spectrum of the so-called reticular seborrheic keratosis and lichen planus-like keratosis (8). As a transition between these two ends of the spectrum, other investigators favor the theory that LCA is a valid concept that should be regarded as part of the spectrum of stucco keratosis and Bowen's disease, at least on histologic grounds (11). The different aspects of these opinions have been summarized in the series entitled "Controversies in Dermatopathology," published in the *American Journal of Dermatopathology* (8–11).

In general, the light-microscopic findings of our cases of LCA were similar to those reported by previous investigators. Transitions of different features were frequently noted within the same lesions. No full-thickness cytologic atypia or significant numbers of mitotic figures were observed. However, based on these results, we cannot meaningfully contribute to the debate on the nosology of LCA, other than to say it has a reproducible histologic spectrum. Instead, the main purpose of this study was to further characterize the DNA ploidy profile of the LCA spectrum and compare it with the information gained from the immunohistochemical detection of proliferating cell nuclear antigen.

Our results confirm that the DNA distribution of LCA is abnormal in all cases examined. The pattern of distribution was variable; it comprised a spectrum from cases having significant (50%) population at DNA index of 1 (normal diploid value) (Fig. 2A), to cases where there is suggestion for peaks at both 1 and 2 (diploid and tetraploid cells) (Fig. 2B–C), to a case with a rather irregular pattern and a wide distribution (Fig. 2D). The only consistent finding was the presence of aneuploid populations, most commonly between the DNA indices of 1 and 2. Although populations at DNA index of 2 (tetraploid cells) could be found in four of seven cases, they were relatively small in two of these cases (~15%) and ~40% in the other cases. This finding is in disagreement with Weinstock's (9) observation of one case of LCA that was found to consist of tetraploid cells. Our results are more in accordance with those of Rabinowitz (10), who examined four cases for DNA content. That study showed two examples of frequency distribution. Some of our cases are similar to his first example; but in his second case, he illustrates a case with a significant (>60%) peak at the DNA index of 2. None of our cases had a >40% population at 2.

Regarding control lesions within other selected diagnostic groups, SK had a single peak at 1, corresponding to normal diploid cells. This pattern was not seen in any of the LCA cases. In contrast, AK and BD had abnormal patterns. AK showed peaks

at 1 and 2, and prominent aneuploid populations, whereas BD seemed to have mostly aneuploid cells. We do not believe that we could distinguish this example of AK from some cases of LCA based on the DNA pattern alone, but BD seemed to have more aneuploidy than most cases of LCA, with the possible exception of case 1 (Fig. 2A).

The mean DNA index (1.44) and range (1.27–1.77) are both comparable to those of previous descriptions (10). This index seems to reflect the predominant populations at 1, 2, and the consistent peak between 1 and 2. Since the mean DNA index does not give information about the distribution pattern, exceeding rates for DNA index of 1, 1.5, and 3 were calculated. These exceeding rates reflected the variable distribution pattern of LCA.

Since 50–80% of the cells are outside the normal diploid value, it is questionable whether these cells are diploid cells in S/G2 phase, or whether they represent abnormal (aneuploid and/or tetraploid) cell clones. Cells in S/G2 phase stain positive with the antibodies to PCNA/cyclin (19–21). The percent of cells staining with this marker was <20% in all seven cases examined; thus, proliferation does not explain the large proportion of nondiploid cells. The tetraploid and aneuploid peaks, therefore, represent cell clones with abnormal DNA content. This lack of proliferation appears to be supported by the low mitotic index observed on light microscopy.

Hyperploidy and aneuploidy can have different significance in various tumors, and DNA ploidy studies are notoriously difficult to reproduce, primarily due to differences in interlaboratory standardization. Despite the extensive use of DNA ploidy studies in other areas of pathology, the results of this technique should not be interpreted outside the context of clinical-pathologic data. Development of a diploid cell line in cell culture for interlaboratory standardization and correlation of the data with cytogenetic chromosome studies will help to improve this methodology. Although our studies support the notion that LCA has abnormal cell populations, the DNA ploidy studies cannot be used as exclusive or indirect evidence for classification or for determination of direction of cell differentiation. On the other hand, these studies cannot exclude either the possibility of a transition into, or the coexistence of, different lesions that can be commonly characterized by large cells on H&E-stained sections, with variable extent of additional changes as seen in solar lentigo, seborrheic keratosis and the bowenoid form of actinic keratoses.

In summary, at least for the time being, the diagnosis of LCA is still based on light microscopy. Despite the low-grade aneuploidy detected by DNA analysis, the lesion carries a benign or indolent biologic behavior which has invariably been observed by investigators. □

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