# RNA, DNA, and Cell Surface Characteristics of Lesional and Nonlesional Psoriatic Skin*

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We have measured the RNA and DNA content and examined cell surface characteristics of human epidermal cells derived from normal skin, and lesional and nonlesional areas of psoriatic skin prior to and following treatment on a modified Goeckerman protocol. Our results show that cells from active psoriatic lesions contain greater numbers of basal keratinocytes when compared with either nonlesional skin from the same patients or skin from healthy volunteers and individuals with other inflammatory skin lesions. Follow-up measurements 2–3 weeks after the initiation of therapy showed that the numbers of basal keratinocytes in resolving psoriatic lesions had decreased and approached normal levels.

Multiparameter RNA/DNA flow cytometric analysis on parallel samples from the same psoriasis patients revealed an increased growth fraction and proportion of cycling cells in both the nonlesional and lesional skin compared with controls. Furthermore, the cellular RNA content was elevated in lesional psoriatic skin when compared with either nonlesional or normal skin. Flow cytometric examination of nonlesional and lesional epidermal cells obtained 2–3 weeks after the commencement of therapy revealed that the growth fraction and mean RNA content of the keratinocytes from resolving psoriatic plaques decreased in response to therapy. In contrast, the proportion of keratinocytes within the S + $G_2$ + M phases of the cell cycle remained elevated. These data indicate that "uninvolved" psoriatic skin exhibits characteristics more closely resembling lesional psoriatic skin than normal skin. The results further suggest that quantitation of cellular RNA content and basal cell number might be sensitive indicators of early treatment response in psoriasis. *J Invest Dermatol 88:646–651, 1987*

Psoriasis is a common chronic proliferative skin disorder characterized by hyperplasia and incomplete and/or abnormal differentiation of the epidermis [1–7]. This disease is clinically manifested as a raised, scaling, erythematous thickening of the skin and can range from small discrete papules to extensive pustular involvement.

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Abbreviations:
AO: acridine orange
EB: epidermolysis bullosa
FBS: fetal bovine serum
FCM: flow cytometric analysis
F530: green fluorescence at 530 nm
F640: red fluorescence at 640 nm
MEM: minimum essential medium
PBS: phosphate-buffered saline
RI: RNA index

We and others have examined the RNA and DNA content of epidermal cells derived from normal skin and lesional and uninvolved areas of psoriatic skin using multiparameter flow cytometric analysis (FCM) of acridine orange (AO)-stained keratinocytes [8,9]. In all 3 types of skin examined, as well as in cultured epidermal cells, FCM analysis of AO-stained epidermal cells was able to resolve 3 subpopulations of keratinocytes that differed in their total cellular RNA content. These 3 subpopulations of low-, intermediate-, and high-RNA content have been designated the "A," "B," and "C" subpopulations, respectively [10]. An increase in the proportion of "B" compartment keratinocytes in skin derived from psoriatic lesions as compared with normal skin has been previously shown [8,9].

The present studies were undertaken to examine the changes that occur in the RNA and DNA content and cell surface characteristics of epidermal cells derived from lesional and uninvolved skin from patients with psoriasis prior to and 2–3 weeks after the commencement of therapy.

## MATERIALS AND METHODS

**Patient Population** Ten patients with psoriasis vulgaris were studied at the Rockefeller University Hospital. This population consisted of 7 women and 3 men with an average age of 48 yrs (age range, 21–76 yrs). Nine patients were treated with a modified Goeckerman protocol of Ultraviolet (UVB) irradiation and coal tar application to the total skin surface (Zetar, Dermik Laboratories Inc., Fort Washington, Pennsylvania). One patient received a treatment regimen of anthralin (Dermal Laboratories, Somerset, New Jersey) and UVB irradiation. Suction blisters were collected

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from patients for examination by cell surface immunofluorescence and flow cytometry immediately prior to commencement of therapy and 2–3 weeks thereafter.

Two types of control populations were studied. The first population consisted of 5 normal volunteers of the same average age and range as the patients with psoriasis studied (average age, 48 yrs; age range, 27–69 yrs). These subjects had no clinical or historical evidence of any dermatologic disorders. The second control population consisted of 7 patients (mean age, 33 yrs; age range, 16–52 yrs) with dermatologic diseases unrelated to hyperproliferation disorders. These included: 2 subjects with functional epidermolysis bullosa (EB), 1 subject with recessive dystrophic EB, 1 subject with EB simplex, 1 patient with subacute cutaneous lupus erythematosus (SCLE), and 2 patients with rheumatoid arthritis. It is important to note that these patients were indistinguishable from healthy subjects by all parameters measured in this study. They are listed in all tables as controls under the subheading "Other Skin Diseases."

**Collection and Processing of Suction Blisters** Informed consent was obtained in compliance with a protocol approved by the Rockefeller University Institutional Review Board. Epidermal suction blisters from patients with psoriasis and control volunteers were generated with a temperature of 38°C and a negative pressure of 280 psi using an apparatus designed by Dr. Alan Lipton and his colleagues of the Electronics Department at the Rockefeller University. In all but 3 cases, suction blisters were collected from the arms of the subjects. Three patients with psoriasis had suction blisters collected from the upper leg. Blister roofs were collected using sterilized forceps and scissors and were placed in sterile phosphate-buffered saline (PBS) (GIBCO, Grand Island, New York) for transportation to the laboratory. We have observed that this procedure results in a split of the epidermis from the dermis within the lamina lucida. Single cell suspensions of keratinocytes were obtained by trypsinization of the epidermal cell sheets as previously described [11]. Fewer than 1% of the cell suspension were melanocytes as determined by Dopa histochemical staining. Epidermal cells derived in such a manner were resuspended in minimum essential medium (MEM; GIBCO) supplemented with 10% fetal bovine serum (FBS; GIBCO), penicillin (100 U/ml) (GIBCO), streptomycin (100 μg/ml) (GIBCO), Fungizone (25 μg/ml) (GIBCO), and hydrocortisone (0.5 μg/ml) (Merck Sharp, and Dome) for subsequent examination by flow cytometry. Alternatively, epidermal cells were resuspended in PBS containing 1% bovine serum albumin (BSA) for subsequent analysis using an indirect immunofluorescence assay with the monoclonal antibody EL-2 as previously described [11].

**Clinical Evaluation of Treatment Response** (Dr. Steven R. Cohen, personal communication). Clinical response to therapy was evaluated by the attending physician based on the following criteria: (1) cutaneous scale: 0 = no discernable scale, 1 = fine (negative auspitz sign), 2 = coarse (positive auspitz sign), 3 = dense (ostraceous); (2) erythema (skin color): 0 = normal flesh tone, 1 = faint pink-red tone, 2 = predominant pink-red tone, 3 = intense red tone; and (3) skin thickness (measured by pinching skin between thumb and index finger): 0 = normal elasticity, 1 = reduced flexibility, 2 = minimal flexibility or inflexible.

**Flow Cytometric Measurement and Analysis** Human keratinocyte suspensions were obtained as described above; 0.2 ml aliquot containing 1–4 × $10^5$ cells in MEM + FBS were mixed with 0.4 ml of 0.08 N HCl, 0.15 N NaCl, and 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, Missouri) at 4°C. Cells were stained 30 s later by the addition of 1.2 ml of a solution containing 0.2 M $Na_2$, $HPO_4$, 0.1 M citric acid buffer pH 6.0, 1 mM disodium EDTA, 0.15 N NaCl, and 6 μg/ml AO (Polysciences, Warrington, Pennsylvania). Under these conditions, interactions of the dye with DNA resulted in green fluorescence with a maximum emission of 530 nm (F530), whereas interactions of the dye with RNA gave red metachromasia at 640 nm (F640). The intensities of these reactions are proportional to the DNA and RNA content, respectively [12,13]. Specificity of staining was evaluated by treatment of the cells with RNase A (Worthington Biochemical Corp., Freehold, New Jersey) or with DNase I (Sigma). Approximately 85–90% of red fluorescence of the epidermal cells were RNase sensitive. However, a subpopulation of cells that has been previously designated as the "C" compartment contained a nonspecific yellowish cytoplasmic fluorescence previously identified as the more differentiated keratinocytes [10].

Fluorescence of individual cells was measured in an FC200 cytofluorograf (Ortho Diagnostic Instruments, Westwood, Massachusetts) equipped with a 488 nm argon ion laser and interfaced to a Data General minicomputer. The red and green fluorescent emissions from each cell were separated optically and the integrated values of the pulses quantitated by separate photomultipliers. Background fluorescence was automatically subtracted; 1 × $10^4$ cells were counted per sample.

**Immunofluorescence** Indirect immunofluorescent staining of epidermal cells with the basal layer/melanocyte-specific monoclonal antibody EL-2 ($IgG_1$) was carried out as previously described on single cell suspensions of viable epidermal cells [11]. Fluorescence was quantitated either by fluorescence microscopy or by flow cytometry in an Ortho System 50H cell sorter. Irrelevant monoclonal antibodies of the $IgG_1$ isotype served as negative controls. Backgrounds of less than 2.0% were observed using the isotype controls.

## RESULTS

The flow cytometric measurement of AO-stained keratinocytes showed 3 distinct subpopulations of cells that differ in RNA content for normal (Fig 1, *left panel*) and psoriatic (Fig 1, *right panel*) skin. We have previously shown by stathmokinetic experiments that each of these subpopulations traverses the cell cycle at different rates [10,14]. The A subpopulation consists of basal keratinocytes with low-RNA content and long cell doubling times (e.g. 120–300 h), whereas the B subpopulation is comprised of larger keratinocytes with higher RNA content and significantly shorter cell doubling times (e.g. 35–40 h). Finally, the C subpopulation comprises the largest, most differentiated keratinocytes that do not divide [9]. We have previously observed these subpopulations in normal skin. We found no differences in the mean RNA content of the A keratinocyte subpopulation from normal vs psoriatic skin. The B and C subpopulation of psoriatic skin, however, appeared to comprise keratinocytes with elevated RNA content. Furthermore, greater numbers of cycling cells were apparent in lesional psoriatic skin when compared with uninvolved skin.

In the present study, we have observed an increase in the number of the more rapidly dividing B keratinocytes in either uninvolved or lesional skin compared with normal skin prior to therapy (Table I, $p < 0.05$). Concomitant with this observation was the presence of decreased numbers of the more slowly dividing A keratinocytes within the psoriatic lesions. The number of B keratinocytes was greatest in skin derived from psoriatic plaques, intermediate in uninvolved skin from the same patients, and lowest in skin from normal volunteers and subjects with nonhyperproliferative skin diseases. Furthermore, as the psoriatic lesions began to resolve in response to therapy, the proportion of B keratinocytes of both lesional and uninvolved skin decreased to within the range of normal skin ($p$ = not significant). The proportion of A keratinocytes also returned to near normal levels after treatment.

Because we found no differences in the mean RNA content of the A keratinocyte subpopulations in normal, uninvolved, and psoriatic skin we sought other means to facilitate the direct comparison of the RNA content of the rapidly dividing B subpopulation of cells. We thus normalized the RNA content of A compartment cells to channel 100, and related the RNA content of compartment B keratinocytes to this value (Table II). We found

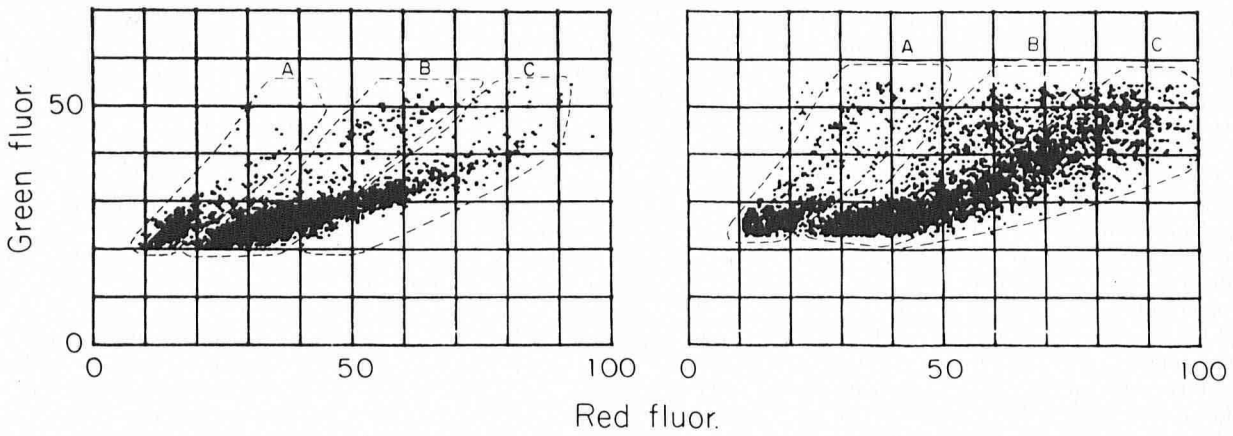


**Figure 1.** Representative RNA (*red fluorescence*)/DNA (*green fluorescence*) cytogram of epidermal cells derived from normal (*left panel*) and psoriatic (*right panel*) skin. Each point on the cytogram represents the RNA (*red*) and DNA (*green*) values of an individual cell. Three subpopulations of keratinocytes can be identified and are marked by the *broken lines*. The low-RNA A subpopulation is similar in both normal and psoriatic epidermis. The higher RNA B subpopulation comprises larger, more rapidly dividing keratinocytes. The B subpopulation keratinocytes from psoriatic lesions (*right panel*) have significantly elevated RNA content (*red fluorescence*) when compared with B keratinocytes from normal skin (*left panel*). Finally, the C compartment contains the largest keratinocytes which do not divide. This subpopulation contains a nonspecific component of AO fluorescence, which is relatively resistent to RNase treatment. Elevated red fluorescence is also observed in C psoriatic keratinocytes (*right*) in comparison to normal keratinocytes (*left*).

no differences in uninvolved psoriatic skin compared with normal skin (or other skin diseases) but observed a significant increase in the mean RNA content of B keratinocytes in lesional psoriatic skin compared with uninvolved skin or skin from control subjects ($p < 0.01$).

Cells were obtained from patients with psoriasis 2–3 weeks after the commencement of therapy. All patients demonstrated greater than 50% decrease in plaque elevation, erythema, and scaling at the time suction blisters were obtained (see *Materials and Methods* for details on clinical evaluation). Analysis of the mean RNA content of the B keratinocytes at this time revealed a decrease in the RNA content of this subpopulation to near normal levels in response to therapy.

We examined the DNA distributions of keratinocytes from normal skin, skin from nonhyperproliferative disorders, and uninvolved and lesional psoriatic skin prior to therapy and 2–3 weeks thereafter (Table III). Significantly increased numbers of cycling ($S + G_2 + M$) cells were observed in epidermal cells derived from both lesional and uninvolved areas of skin compared with control skin ($p < 0.05$). In addition, in 8 of 10 patients studied, keratinocytes from the psoriatic plaque contained greater numbers of cycling cells ($S + G_2 + M$) than did keratinocytes derived from an uninvolved area of skin from the same patient. Slight decreases in the mean number of cycling cells were observed in epidermal cell populations derived from both uninvolved and lesional skin 2–3 weeks after the commencement of therapy. These differences, however, were not significant when either uninvolved pretherapy and posttherapy or lesional pretherapy and posttherapy samples were analyzed in a paired Student's *t*-test. This is in contrast with the significant decrease in RNA content observed within cells from the same samples.

Finally, we have examined the cell surface characteristics of epidermal cells derived from normal skin or from lesional and uninvolved skin from patients with psoriasis using the monoclonal antibody EL-2, which is specific for epidermal basal keratinocytes and melanocytes [11]. Indirect immunofluorescence studies with EL-2 on single-cell suspensions of keratinocytes obtained from lesional and uninvolved skin from patients with active psoriasis before and approximately 2–3 weeks after the initiation of medical therapy are shown in Table IV. The mean proportion of EL-2 reactive keratinocytes from active psoriatic plaques was 54.1 ± 5.0%, which was significantly increased when compared with the mean obtained from uninvolved skin from the same patients (35.1 ± 5.0%; $p = 0.01$) and skin from control individuals (29.6 ± 2.7 and 30.5 ± 3.6 respectively; $p = 0.0025$). After 2–3 weeks of therapy, the mean proportion of EL-2 reactive keratinocytes in lesional skin dropped to 38.5 ± 5.0%, which was significantly less than the mean obtained from active psoriatic plaques ($p = 0.05$), and was almost identical to the percentage obtained from posttreatment uninvolved skin.

One of the patients enrolled in this study is a 50-year-old man with a 28-year history of psoriasis treated in the past with tar, topical steriods, and a 7-year course of psoralen and UVA with minimal lasting improvement. He was first admitted to the Rockefeller University Hospital in July, 1985 for treatment of severe psoriasis. At the time, there were widespread erythematous hyperkeratotic plaques over his arms, legs, and sacral region. Prior to treatment, skin biopsies and suction blisters were obtained.

**Table I.** The Proportion of Rapidly Dividing B Subpopulation Keratinocytes Is Increased in Active Psoriatic Plaques

| | Proportion of B Keratinocytes (Mean % ± SEM) | | | |
|---|---|---|---|---|
| | Controls | | Psoriasis | |
| | Normal Skin (n = 5) | Other Skin Diseases (n = 7) | Nonlesional Skin (n = 10) | Lesional Skin (n = 10) |
| Pretherapy | 63.8 ± 2.7 | 51.0 ± 6.7 | 72.8 ± 3.8 | 76.7 ± 4.0 |
| Posttherapy | NA[a] | NA | 63.1 ± 5.4 | 65.3 ± 1.7 |

[a]NA = not applicable.

**Table II.** The Mean RNA Content of the B Subpopulation of Keratinocytes Is Increased in Active Psoriatic Plaques

| | Mean RNA Content of the B Subpopulation of Keratinocytes (Mean F > 640 ± SEM) | | | |
|---|---|---|---|---|
| | Controls | | Psoriasis | |
| | Normal Skin (n = 5) | Other Skin Diseases (n = 7) | Nonlesional Skin (n = 10) | Lesional skin (n = 10) |
| Pretherapy | 170.0 ± 10.4 | 170.0 ± 9.0 | 179.0 ± 13.1 | 232.4 ± 15.0 |
| Posttherapy | NA[a] | NA | 173.4 ± 5.1 | 190.6 ± 12.0 |

[a]NA = not applicable.

The RNA index of the B keratinocytes from the psoriatic lesion before treatment was 317, 27% of the cells were cycling, and 60% of the cells reacted with EL-2. Thus, all of the parameters measured prior to therapy fell outside of the normal range.

He was treated with a modified Goeckerman protocol for 4 weeks with considerable flattening and lightening of his psoriatic plaques. After 3 weeks of therapy, repeat suction blisters were obtained. Although a decrease in RNA index (RI) (posttherapy RI = 223) and the percent of cycling cells (21%) was apparent after treatment, neither of these parameters returned to within the normal range. Furthermore, a slight increase in the percentage of EL-2 keratinocytes (62%) was observed at this time. Therefore by all of the parameters measured, the patient had not achieved remission at the time of follow up. The patient stopped treatment at this time and experienced a relapse within 1 month after the discontinuation of therapy.

In December, 1985, he was readmitted to the Rockefeller University Hospital with hyperkeratotic plaques and widespread eruptions involving most of the upper and lower extremities, abdomen, and back. Repeat suction blisters were obtained from 1 uninvolved area of skin and 2 separate lesional sites. The RI of the 2 lesions were 238 and 234, respectively. Furthermore, the percent of cycling cells were 20% and 29%, respectively, indicating that the active plaques showed very similar RNA/DNA characteristics. When the percentage of EL-2 reactive cells was quantitated, 74% of the lesional keratinocytes bound the antibody. The results from this patient indicate that RNA/DNA flow cytometric measurements of keratinocytes from 2 different psoriatic plaques were very similar. The patient is currently being treated with a modified Goeckerman protocol and will be followed up on a long-term basis.

## DISCUSSION

In the present study, we have compared the RNA/DNA and cell surface characteristics of skin from control subjects with lesional and uninvolved skin from patients with active psoriasis prior to and 2–3 weeks following the initiation of therapy.

The immunofluorescence studies we have performed using the basal layer specific monoclonal antibody EL-2 indicate that greater numbers of basal cells can be detected in active psoriatic plaques in comparison to either uninvolved skin from the same patient or healthy skin from control individuals. This observed increase in the number of germinative cells confirms the work of others using a different panel of monoclonal antibodies [15]. It has been shown that epidermis from active psoriatic lesions has deeper rete pegs than normal skin, with a concomitant loss of stratum granulosum. Furthermore, an increase in the number of basal-like cell layers in psoriatic epidermis has also been reported [2]. The increased reactivity of psoriatic epidermis with the monoclonal antibody EL-2 may reflect both the change in geometry of the psoriatic epidermis and the increased numbers of basal layers observed. It has also been shown that the epidermis from psoriatic plaques reverts to a more normal morphologic appearance as they begin to resolve. Our data support these observations in that as patients responded to therapy, the number of EL-2 reactive basal keratinocytes from the psoriatic plaques decreased and approached the levels detected in normal skin from control individuals.

We have previously shown that multiparameter RNA/DNA analysis of human keratinocytes can discriminate 3 subpopulations of cells characterized by either low (A), intermediate (B), or high (C) RNA content [10]. Stathmokinetic studies have shown that the low-RNA A cells have significantly longer generation times than high-RNA B keratinocytes. Using the AO technique, it has been shown that epidermal cells derived from both uninvolved skin and psoriatic lesions had increased numbers of cells within the more rapidly proliferative B compartment [8,9]. Of significance in this study has been the observation of elevated levels of cellular RNA content in the B subpopulation of epidermal cells derived from psoriatic lesions. This increase in RNA content is indicative of an unbalanced growth state in which cells accumulate greater amounts of RNA than they would otherwise normally accumulate [16]. It is important to note that this unbalanced growth is not observed in the low-RNA A subpopulation, but in the more rapidly dividing B subpopulation. We have previously shown a strong correlation between cellular RNA content and the rate of cell cycle traverse; in particular, the rate of S phase traverse [17,18]. We have observed in a number of different systems that cells in the $G_1$ phase of the cell cycle that contain high amounts of cellular RNA traverse S phase more

**Table III.** The Number of Cycling (S + $G_2$ + M) Epidermal Cells Is Increased in Active Psoriasis and Uninvolved Skin in Comparison to Normal Epidermis

| | Number of Cycling Cells (Mean % S + $G_2$ + M ± SEM) | | | |
|---|---|---|---|---|
| | Controls | | Psoriasis | |
| | Normal Skin (n = 5) | Other Skin Diseases (n = 7) | Nonlesional Skin (n = 10) | Lesional Skin (n = 10) |
| Pretherapy | 10.1 ± 2.9 | 12.6 ± 2.6 | 20.6 ± 3.4 | 24.5 ± 3.1 |
| Posttherapy | NA[a] | NA | 19.3 ± 2.3 | 22.5 ± 1.1 |

[a]NA = not applicable.

**Table IV.** The Number of EL-2 Reactive Keratinocytes Is Increased in Active Psoriatic Plaques

| | Proportion of EL-2 Reactive Cells (Mean % Positive ± SEM[a]) | | | |
|---|---|---|---|---|
| | Controls | | Psoriasis | |
| | Normal Skin (n = 5) | Other Skin Diseases (n = 7) | Nonlesional Skin (n = 10) | Lesional Skin (n = 10) |
| Pretherapy | 29.6 ± 2.7 | 30.5 ± 3.6 | 35.1 ± 5.0 | 54.1 ± 5.0 |
| Posttherapy | NA[b] | NA | 38.3 ± 5.0 | 38.5 ± 5.0 |

[a]Quantitated by indirect immunofluorescence using tetra-methyl-rhodamine isothiocyanate-conjugate F(ab)'2 fragments of goat antimouse IgG antiserum (TAGO, Burlingame, California). A minimum of 200 cells per sample was counted by fluorescence microscopy.
[b]NA = not applicable.

rapidly than $G_1$ phase cells with lower cellular RNA content [15,16]. The appearance of a keratinocyte subpopulation in psoriatic skin with elevated cellular RNA content would therefore be consistent with the shorter keratinocyte doubling times seen in psoriatic lesions using autoradiographic techniques [7].

A decrease in the B compartment RNA content to quasinormal levels was observed in patients who responded to therapy. By contrast, keratinocytes from the patient who experienced a severe relapse within 1 month after cessation of treatment did not show a reduction in RNA content to near normal levels. These results suggest that measurement of cellular RNA content might be a sensitive marker in monitoring the response to therapy in patients with psoriasis and may be predictive of clinical susceptibility to relapse.

In contrast to the decrease observed in cellular RNA content during successful response to therapy, the number of cycling (S + $G_2$ + M) psoriatic epidermal cells from both uninvolved and lesional skin remained elevated in comparison to that of normal control skin. Moreover, in 3 of 10 patients examined, an increase in the proportion of cycling cells was observed in the psoriatic lesions 2–3 weeks following the commencement of successful therapy. Thus single parameter measurement of DNA content in keratinocytes from psoriatic lesions is not always an accurate reflection of the clinical status of the disease.

Taken together, these data indicate that the evolution of a psoriatic plaque involves the induction of an unbalanced growth state within the more rapidly dividing B subpopulation of keratinocytes as evidenced by an accumulation of RNA. Based on our previous observations on the correlation between RNA content and the rate of cell cycle traverse, we would expect that the increased RNA content in the B subpopulation of keratinocytes should correlate with a shorter cell doubling time of this subpopulation. Thus, our observations support the hypothesis that the clinical manifestation of psoriasis most likely reflects both an increase in growth fraction (B compartment cells) as well as a shorter cell cycle doubling time. These studies also indicate that both EL-2 reactivity and RNA content are more sensitive markers of treatment response than is DNA content, in that changes in cellular RNA content and EL-2 antibody binding are observed prior to any significant changes in the DNA profiles of psoriatic lesions during treatment regimens.

The present studies also clearly indicate that uninvolved skin from patients with active psoriasis showed abnormalities in the growth fraction, percentage of cycling cells, and in the proportion of EL-2 reactive keratinocytes when compared with control skin from normal volunteers or individuals with nonhyperproliferative skin disorders. Although the cellular RNA content from nonlesional skin remained relatively low in 9 of 10 psoriasis patients, the 1 patient who suffered severe relapse within 1 month after cessation of therapy exhibited significantly elevated RNA content in the epidermal cells from uninvolved skin (RI = 217). The possible role of increased cellular RNA content in the development of an active psoriatic lesion is presently under investigation.

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