Monoclonal Antibodies for Epidermal Population Analysis*

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Three keratin antibodies (RKSE 60, Clone 77, and a rabbit polyclonal) and 2 vimentin antibodies (Vim ab and a rabbit polyclonal) were investigated using frozen sections of normal and psoriatic skin. Of these, the monoclonals RKSE 60 and Vim ab were selected for quantitative population analysis of healthy epidermis, psoriatic uninvolved epidermis, and psoriatic lesions.

Suspensions of isolated cells were prepared from biopsy specimens by trypsinization, and stained with RKSE 60 or Vim ab using an indirect immunofluorescence assay. Our results showed an increase in the germinative fraction from the normal value of 30% to almost 50% in the psoriatic lesion; in absolute terms this corresponds to a 6-fold increase in the size of the germinative compartment. More interesting, the germinative psoriatic uninvolved epidermis (38%) was also significantly higher than normal. The percentage of vimentin-positive cells (Langerhans cells and melanocytes) was nearly double that of normal in both the lesion and the uninvolved psoriatic epidermis. We conclude that, in contrast to statements frequently encountered in the literature, the “uninvolved” skin of the patient is morphologically and functionally different from that of the healthy individual. J Invest Dermatol 87:72–75, 1986

The epidermis is not homogeneous in its cellular composition. Apart from keratinocytes, other cell types are present such as Langerhans cells, melanocytes, and in pathologic situations also inflammatory cells. The keratinocytes themselves are functionally heterogeneous, ranging from germinative cells (cycling or resting) to those at the terminal, fully differentiated state. Epidermal growth control is achieved by a number of complex mechanisms that as yet are only partly understood. A proper identification and quantification of the epidermal cellular constituents is obviously a prerequisite for the elucidation of these interactions.

In this paper we describe the quantitative differentiation of epidermal cell types in cell suspensions from healthy controls, psoriatic uninvolved skin, and from lesions by using antisera specific for individual cell types.

MATERIALS AND METHODS

Patients and Biopsy Procedure Seven patients with chronic plaque psoriasis were selected from those attending the Outpatient Department (age range, 25–61 years). None had received therapy for any kind for at least 2 weeks. The 6 healthy control subjects were paid volunteers (age range, 20–40 years) with no sign or history of skin disease.

Biopsies were cut using a razor blade in conjunction with a metal guard [1]: these averaged 3 mm diameter and about 0.4 mm thickness. As far as possible 2 biopsies were taken from the shoulder and 2 from the outer aspect of the forearm (about 5 cm distal to the elbow) in all controls and the uninvolved skin of all psoriatic patients for routine preparation of cell suspensions; in the latter group these were at least 5 cm distant from any lesion. At least 1 biopsy of lesional skin was also taken from similarities on each patient. Some additional biopsies were also taken from controls, uninvolved skin of patients, and from lesions for the preparation of frozen sections.

The prior consent of the Ethics Committee of this University was obtained for these experiments.

Frozen Sections Skin biopsies were washed in saline and embedded in Ames OCT compound (Ames Company, Indiana, U.S.A.) in the cryostat at −20°C. Sections of 6 μm were cut and air dried. For direct use these were handled as described below. For storage, the air-dried sections were fixed in acetone and kept at 4°C until use.

Preparation of Cell Suspensions Skin biopsies were incubated for 20 min at 37°C in 0.2 ml 0.15 M sodium phosphate buffer, pH 7.6, containing 1% trypsin (Difco 1:250) and 0.3% dithioerythritol (Sigma) [2]. After incubation, the intact specimens were transferred to a test tube with 0.5 ml ethanol 70% (−20°C). After 2–5 s sonication at 70 W (Sonifier B12, Branson Sonic Power Company) with the tip of the vibrator as close as possible to the biopsy, the epidermis detached from the dermis and the epidermal cells dissociated. The stratum corneum and the dermis remained intact with this procedure, so that a single cell suspension was obtained with no admixture of dermal cells. Ethanol 70% was added to a final volume of 2 ml. This was filtered through gauze (mesh 50 μm, Phyne, F.R.G.) and stored at −20°C until use.

Antisera The following antisera were used in this study:

* A preliminary report of this work was presented at the 14th Annual Meeting of the European Society for Dermatological Research, Amsterdam, The Netherlands, May 20–23, 1984.

** Abbreviations:
FITC: fluorescein isothiocyanate
IF: indirect immunofluorescence
PBS/CS: phosphate-buffered saline/calf serum
TRITC: tetramethylrhodamine isothiocyanate

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preparation and specificity testing of these sera have been described elsewhere [3,4]. Preliminary experiments showed that Clone 77 and RKSE 60 gave identical results; the latter was selected for routine analysis because of its commercial availability (Euro-Diagnostics, B.V., Apeldoorn, N.L.).

**Antivimentin Sera:** Two types of antisera were used. The first was a rabbit antiserum raised against vimentin isolated from calf lens [5]. The second was a monoclonal antibody (Vim ab) directed against calf lens vimentin [6] (Euro-Diagnostics B.V.). The rabbit antiserum was used only for the double-labeling experiments (below); the monoclonal was employed for all routine measurements.

**Labeled Antibodies:** Goat antiamouse IgG (GAM) and goat antirabbit IgG (GAR) labeled with fluorescein isothiocyanate (FITC) or tetramethylrhodamine isothiocyanate (TRITC) (Nordik, Tilburg, N.L.) were used in the indirect immunofluorescence (IF) assay.

**Indirect Immunofluorescence Staining** Air-dried cryostat sections were stained for IF as described elsewhere [4]. The sections were viewed with a fluorescence microscope with epifluorescent illumination, sometimes using a phase contrast system.

Ethanol-fixed suspensions derived from a single biopsy were centrifuged at 800 g for 3 min. The ethanol was discarded and the sediment resuspended in 1 ml acetone. The suspensions were pelleted at 800 g and resuspended in 1 ml phosphate-buffered saline containing 5% calf serum (PBS/CS); this was then divided into 2 parts. These were again centrifuged and the 2 pellets resuspended in the incubation mixtures. One part of the cell suspension (A) was incubated in 0.2 ml PBS/CS with RKSE 60, the other part (B) with Vim ab in appropriate dilutions. The cells were incubated at room temperature for 30 min with gentle stirring, washed twice with PBS/CS, and resuspended in PBS/CS containing the appropriate second antibody labeled with FITC. After 45 min further incubation, the cells were washed twice in PBS/CS and resuspended in 0.2 ml PBS/CS.

Double-labeling experiments were carried out in order to investigate the possible expression of vimentin and mature keratin by the same cell. The suspensions were handled as described above except that the first incubation was performed simultaneously with RKSE 60 and vimentin antiserum and the second incubation simultaneously with GAM TRITC and GAR FITC.

**Microscopic Quantification of the Subpopulations** The suspensions A and B derived from a single biopsy were analyzed in the following way.

**Suspension A:** The fraction of RKSE 60-positive cells was determined in a total number of about 400 cells. These were considered as differentiated keratinocytes, the negative cells as a mixture of germinative keratinocytes and nonkeratinocytes.

**Suspension B:** The fraction of vimentin-positive cells was counted in a total number of about 400 cells. These were considered to be nonkeratinocytes.

From the figures derived from suspensions A and B, the fraction of the germinative cells was calculated as follows:

\[
\text{% germinative cells} = 100\% - \text{Vim ab}(+)\text{ cells} - \% \text{RKSE 60}(+)\text{ cells.}
\]

**RESULTS**

**Frozen Sections** Fig 1 illustrates staining patterns shown by the various antibodies against normal human skin. In Fig 1A, using the polyclonal rabbit antiserum against skin keratins, the entire epidermis is stained. Using RKSE 60, however, it is seen that the basal cell layer (and in some areas the adjacent suprabasal layer) remains unstained; this is the population that is considered to be the germinative pool [7]. This suggests strongly that this monoclonal is directed only against differentiated keratinocytes. Fig 1C illustrates the results obtained using Vim ab; dendritic staining is apparent in the epidermis and more general staining of cellular elements in the dermis. This is compatible with the distribution of mesenchymal cells in the skin.

Fig 2 shows psoriatic lesion skin stained with the same 3 antibodies. Comparing Fig 2B with Fig 2A, it is seen that a considerably greater number of cells are RKSE 60-negative; this
was especially marked in the lower portions of the rete ridges, where 3-4 unstained layers were sometimes observed. The demarcation between positive and negative cells corresponds well with the accepted boundary of the germinative population in the psoriatic lesion [8,9]. Fig 2C illustrates the pattern of Vim ab staining in the lesion; the distribution in the epidermis seems qualitatively similar to the normal picture.

No qualitative differences could be observed between stained sections of psoriatic uninvolved and healthy control skin.

**Cell Suspensions** Positive and negative cells could easily be distinguished in preparations of isolated cells. It was clear from double-labeled suspensions that vimentin-positive cells were always negative to RKSE 60. Table I summarizes the data obtained from the 2 sites in healthy controls and the psoriatic uninvolved skin. Two conclusions may be drawn from these data; first, that there are marked differences between the healthy and the psoriatic uninvolved specimens, and second, that the selection of site does not significantly influence the results (elbow vs shoulder in all cases p > 0.15. Student's t-test). The data from these 2 anatomic sites were therefore pooled for subsequent statistical analysis.

In Table II we compare the pooled data for the healthy and uninvolved epidermis with the values derived from 8 psoriatic lesions. The overall distributions are significantly different for all 3 groups of specimens at the level p < 0.001 (one-way analysis, F-test). Further statistical evaluation of the individual subpopulations (Duncan test) showed significant differences between all groups for RKSE 60-positive cells and for the germinative pools. In the case of the vimentin-positive cells, both uninvolved and lesional epidermis of psoriatics were increased (p < 0.02) but there was no significant difference between these latter groups.

Remarkably, in the 6 patients from whom we obtained both uninvolved and lesional biopsies, there was a high correlation between the numbers of Vim ab-positive cells in these 2 specimens (Fig 3) (Pearson correlation coefficient is 0.87, p < 0.02).

**DISCUSSION**

In this study we have analyzed epidermal cell suspensions by the use of antisera that specifically recognize certain cell types. Clearly, in the analysis of a population into 3 subgroups (mesenchymal cells, differentiated keratinocytes, and germinative keratinocytes), only 2 require positive identification, the third being quantified by difference. Here we chose the monoclonals Vim ab and RKSE 60 for identification of mesenchymal cells and differentiated keratinocytes, respectively, since these antibodies are commercially available, are well-documented in their specificities, and because we had prior experience in their use. Preliminary experiments using Pab601 (a marker for germinative cells) in conjunction with flow cyrometric analysis confirmed that our present estimates of the germinative pool are very similar to values obtained by direct staining (Bauer, unpublished data).

For quantitative investigations, however, it is obvious that cell counting of populations in histologic sections is tedious, often nonreproducible, and liable to subjective errors. For this reason the population analysis reported here is based on suspensions of isolated cells obtained by trypsinization of microbiopsies. It has already been demonstrated by us [2,10] that this procedure results in a uniformly high yield of all viable cell types; microscopic observation of the residual dermis and stratum corneum shows few or no adherent cells.

The interpretation of Vim-positive cells presents no difficulty; clearly these are cells of mesenchymal origin, i.e., Langerhans cells or melanocytes [5,6,11]. This is in line with the dendritic appearances of Vim-positive cells reported here. However, the exact definition of RKSE-positive cells requires more caution. It is clear that keratinocytes that have left the germinative pool (i.e., can no longer divide) are characterized by the production of specific keratin polypeptides [12], and also that monoclonals such as RKSE 60 react with antigenic determinants on these molecules (nr.10, Moll catalogue, personal communication, F. C. S. Ra-

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**Table I.** Summary of Indirect Immuno-Fluorescence Studies of Cell Suspensions Derived from Healthy Controls and Psoriatic Uninvolved Epidermis

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Biopsies (n)</th>
<th>Site</th>
<th>RKSE 60+ (%)</th>
<th>Vim ab+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy epidermis</td>
<td>12</td>
<td>Elbow</td>
<td>65.9 ± 6.5</td>
<td>4.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Shoulder</td>
<td>63.0 ± 3.0</td>
<td>6.2 ± 2.9</td>
</tr>
<tr>
<td>Psoriatic uninvolved epidermis</td>
<td>12</td>
<td>Elbow</td>
<td>52.3 ± 7.8</td>
<td>9.3 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>Shoulder</td>
<td>52.5 ± 7.7</td>
<td>10.0 ± 5.6</td>
</tr>
</tbody>
</table>

Figures are means ± SD.

**Table II.** Comparison of Values from Psoriatic Lesional Epidermis with Pooled Data for Healthy Controls and Psoriatic Uninvolved Epidermis

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Biopsies (n)</th>
<th>RKSE 60+ (%)</th>
<th>Germinative (%)</th>
<th>Vim ab+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy epidermis</td>
<td>24</td>
<td>64.5 ± 5.2</td>
<td>30.0 ± 5.7</td>
<td>5.5 ± 2.5</td>
</tr>
<tr>
<td>Psoriatic uninvolved epidermis</td>
<td>24</td>
<td>52.4 ± 7.6</td>
<td>37.9 ± 8.8</td>
<td>9.7 ± 4.9</td>
</tr>
<tr>
<td>Psoriatic lesion</td>
<td>10</td>
<td>41.3 ± 12.0</td>
<td>48.5 ± 13.4</td>
<td>10.2 ± 3.8</td>
</tr>
</tbody>
</table>

Figures are means ± SD.
maekers). Here we use the term "differentiated" for this RKSE 60-positive keratinocyte population. The existence of a non-differentiating but RKSE 60-negative population seems unlikely in view of the complimentary staining patterns of Pab601 and RKSE 60 [13]. The finding that some differentiation markers may be expressed by keratinocytes in S phase [14] does not apply for markers recognized by RKSE 60 since the occurrence of RKSE 60-positive cells in the basal layer was never observed. However, the converse possibility, that an RKSE 60-positive cell in a suprabasal position is still able to divide, cannot be excluded with certainty. None of these arguments of course exclude the possibility that the germinative pool is heterogeneous, containing for example stem cells and amplification cells.

As expected, we found a gross increase in the fraction of germinative cells in the psoriatic lesion [8,9]. It may be noted that, since the total epidermal volume per unit area of skin surface is approximately 4 times greater than normal in the lesions [9], we may calculate an absolute increase of about 6-fold in the germinative cell population. However, a far more striking and interesting observation was that the germinative fraction is also markedly raised in the uninvolved epidermis of the patients, rising from less than one-half of the differentiated pool in the controls to just over two-thirds in the patients. It should be emphasized that the selection of biopsy sites (careful clinical examination and a minimum of 5 cm distance from any lesion) precluded the possibility that these samples contained lesional material. The concept of an increased germinative pool in the uninvolved epidermis has already been suggested by us as an explanation of the raised values for percentage S and G3M cells compared to healthy controls [15]. This finding is also compatible with biochemical observations which have, from time to time, appeared in the literature; an example is the increased oxygen consumption and CO2 production by psoriatic uninvolved epidermis [16].

Another interesting finding is the increased density of vimentin-positive cells in the psoriatic epidermis (both lesional and uninvolved). Since these cells are a mixture of Langerhans cells and melanocytes we cannot say with certainty which of these is in fact altered. However, an increase of melanocytes seems to be unlikely since these patients were not subjected to UV radiation therapy. The data from Fig 3 indicate that, although there is a wide variation in the percentage of vimentin-positive cells among different psoriatic patients, the density in the lesion remains equal to that in the uninvolved skin. This suggests that this feature is a genetic characteristic of the individual patient rather than a local consequence of the disease process.

In summary, we may conclude that our present data confirm and extend previous observations regarding the cellular composition of the psoriatic lesion. In addition, we report the new and important finding that the "uninvolved" epidermis of the patient is morphologically and functionally different from that of the healthy person. These data may lay a firmer basis for the interpretation of future observations at the cellular and molecular levels.

The histological assistance of Oluf Moecker of the Department of Pathology, Nijmegen was highly appreciated. We would like to thank Gees van Muijen of the Department of Pathology, Leiden for providing us with Clone 77 and Mrs. Birgit Lane of the London Hospital, London for PAB601.

REFERENCES