Identification of Pigment Cell Antigens Defined by Vitiligo Antibodies

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Patients with vitiligo have circulating antibodies to pigment cells. To characterize this response further and to identify the antigens defined by vitiligo antibodies, sera of 23 patients with vitiligo and 22 patients with unrelated conditions were analyzed by immunoprecipitation and SDS-PAGE analysis of 125I-labeled cell antigens on pigment and control cells. Antibodies to pigment cell antigens were present in 18 (78%) of the patients with vitiligo but in only three (14%) of the control patients (p < 0.05). The antibodies were directed to one or more antigens with molecular weight (MW) in kilodaltons (kD) of approximately 35, 40–45, 75, 90, or 150. The responses were most commonly directed to the 40–45-kD, 75-kD, and 90-kD antigens. Antibodies to these antigens were present in 74%, 57%, and 35% of vitiligo patients versus in 14%, 9%, and 0% of control individuals. The 35-kD and 90-kD antigens were preferentially expressed on human pigment cells, whereas the 40–45-, 75-, and 150-kD antigens were expressed on both pigment and control cells. These antigens were labeled by the lactoperoxidase technique, suggesting that they are cell surface antigens. These results confirm that antibodies to pigment cells are associated with vitiligo. These antibodies are directed to several cell surface antigens, some of which are preferentially expressed on pigment cells. J Invest Dermatol 98:162–165, 1992

Vitiligo is a disease in which melanocytes are selectively destroyed; its etiology is unknown. However, the findings that there are antibodies to pigment cells in sera of patients and of animals with vitiligo [1–7], that there is a correlation between the presence and level of these antibodies and the extent [6] and activity of vitiligo [7], and that these antibodies can selectively kill human melanocytes in vitro [8,9] suggest that this disease is an autoimmune process. This study was conducted to identify the pigment cell antigens defined by antibodies in patients with vitiligo and to examine their specificity.

MATERIALS AND METHODS

Sera Sera were obtained from 23 patients with vitiligo and 22 individuals with unrelated skin diseases, who were matched for age, race, and gender.

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Abbreviations:
BPE: bovine pituitary extract
EDTA: ethylenediamine tetraacetic acid
kD: kilodalton
MEM: minimum essential medium
MW: molecular weight
PBS: phosphate-buffered saline
PMA: phorbol myristate acetate
SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis
TCA: trichloroacetic acid

Cells Studies were performed on a panel of pigment cells including human melanocytes, human pigmented (SK-mel-23, SK-mel-30) and non-pigmented melanoma (SK-mel-28, M20 and M14) cells, xenogeneic murine B16 melanoma, and hamster (HM54) melanoma. Control cells included erythroleukemia (K562), colon carcinoma (SK-CO-1), lung carcinoma (A549), rhabdomyosarcoma (RD), baby hamster kidney (BHK-1), and normal human lymphocyte. M20 and M14 melanoma were provided by Dr. Donald Morton (UCLA, Los Angeles, CA) and HM54 by Dr. G. Lipkin (NYU School of Medicine, New York, NY). Human melanocytes (Clonetics, San Diego, CA) were cultured in melanocyte growth medium containing basic fibroblast growth factor (BFGF 1 ng/ml), bovine pituitary extract (BPE 0.2% v/v), insulin (bovine 5 µg/ml), hydrocortisone (0.5 µg/ml), phorbol myristate acetate (PMA, 10 ng/ml). To evaluate the effect of PMA on the expression of antigens, a batch of melanocytes was washed exclusively and cultured in the conditions with and without PMA for 8 d before being labeled. Normal human peripheral blood lymphocytes were obtained and separated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) on the same day as used. All other cells were obtained from the American Type Culture Collection (Rockville, MD) and cultured in minimum essential medium (MEM) (Whittaker, Walkersville, MD) supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY), one times the non-essential amino acids and antibiotics.

Lactoperoxidase-Catalyzed Radiiodination Surface macromolecules on pigment and control cell were radiiodinated by the lactoperoxidase technique [10], washed three times with phosphate-buffered saline (PBS) with 0.02% sodium iodide, and solubilized with 1 ml of lysing buffer (0.5% NP-40, 0.02% NaCl, and 0.025 M Na2 ethylenediamine tetraacetic acid [EDTA]). The insoluble material was removed by centrifugation at 12,000 × g for 20 min. Radioactivity associated with labeled macromolecules was measured by precipitation with 10% trichloroacetic acid (TCA).

Immunoprecipitation and SDS-PAGE Analysis This was performed as previously described [10]. Ten microliters of radioiodinated cell extract were added to 50 µl of patient serum in a total volume of 80 µl in a microtiter plate. After 16 h at 4°C, the precipitins were separated by SDS-PAGE. The gels were dried and autoradiographed and analyzed by densitometry.
Table I. Incidence of Antibodies to Pigment Cell Surface Antigens

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number</th>
<th>With Antibodies to Pigment Cell* Number (%)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitiligo</td>
<td>23</td>
<td>18 (78)</td>
<td>0.05</td>
</tr>
<tr>
<td>Control</td>
<td>22</td>
<td>3 (14)</td>
<td></td>
</tr>
</tbody>
</table>

* SK-mel-28 melanoma cell.

Dinitrated cell lysate adjusted to contain approximately 200,000 insoluble TCA counts per minute were incubated with 10 µl of undiluted sera for 16 h at 4°C. Bound antigens were precipitated with protein A-Sepharose (Pharmacia, Uppsala, Sweden), washed thoroughly, run on SDS-8% PAGE, and visualized by autoradiography. Presence of antibodies to a pigment cell antigen was shown by the presence of a labeled band in the autoradiograph.

RESULTS

Sera of 23 patients with vitiligo and 22 control individuals were tested for antibodies to pigment cell antigens by immunoprecipitation and SDS-PAGE analysis. The cell line used as a target was SK-mel-28 melanoma cells. The results are shown in Table I. Eighteen (78%) of the patients with vitiligo had antibodies to one or more pigment cell antigens. Similar antibodies were detected in only three (14%) of control sera (p < 0.05).

By SDS-PAGE and autoradiographic analysis of the immunoprecipitate, the antibodies in patients with vitiligo were directed to various patterns of one or more antigens with approximate MW of 150, 90, 75, 40–45, or 30 kD (Fig 1, lanes A–L). The antibodies were most commonly directed to the 40–45 kD antigen (in 74% of patients), then to the 75 kD and 90 kD antigens (in 57% and 35% of patients respectively) (see Table II). In control sera, the antibodies were directed to the 40–45 kD (in 14% of patients) or the 75 kD antigens (in 9% of patients).

To study the specificity of the antigens defined by antibodies in patients with vitiligo, 5 antibody-positive vitiligo sera were used to study the expression of these antigens on a panel of pigment and control cells. The results are summarized in Table III and illustrated in Fig 2. The 90-kD antigen was strongly expressed on human melanocytes and more weakly on three of five human melanoma, but only on 1 of 8 control cell lines. The 35-kD antigen was only expressed on SK-mel-28. The 75-kD and 40–45-kD antigens were commonly and strongly expressed by most pigment and control cells. The 150-kD antigen was expressed on 3 of 5 (60%) human pigment cell lines and 4 of 8 (50%) control cells.

To exclude the possibility that the expression of these antigens on melanocytes was induced by culture conditions, especially PMA, replicate plates of human melanocytes were cultured in melanocyte growth medium with or without PMA for 8 d, labeled under identical conditions, and tested for the presence of antigens by immunoprecipitation SDS-PAGE analysis using sera of four antibody-positive vitiligo sera. The pattern and density of antigens defined by the vitiligo sera on both sets of cells were identical, indicating that the expression of these antigens was unrelated to the presence of PMA.

DISCUSSION

The most important finding of this study is that some of the pigment cell antigens defined by antibodies in patients with vitiligo have been identified as antigens with MW of approximately 150, 90, 75, 40–45, and 35 kD. These antigens were iodinated by lactoperoxidase technique, which labels preferentially the cell surface antigens, suggesting that they may be expressed on the surface of pigment cells. Also, antigens labeled by metabolic methionine cannot be identified by vitiligo antibodies.
interesting antigen was the 90-kD antigen, antibodies to which were present in 35% of patients with vitiligo and in none of the control individuals. This antigen was selectively expressed on human pigment cells. It was expressed on 4 of 6 human pigment lines, but on none of 2 xenogeneic melanoma cell lines. By contrast, it was expressed on only 1 of 6 other control cell lines. The 35-kD antigen was expressed by only one of the human pigment cells and on none of the controls; however, antibody to it was present in only 4% of the patients with vitiligo and in none of the controls. The 75-kD and 40–45-kD antigens, antibodies to which were present in 57% and 74% of patients with vitiligo and 9% and 14% of control individuals, respectively, were common tissue antigens as they were expressed on most pigment and control cells. It is of interest that the pigment cell antibodies present in some normal individuals were directed to these two antigens. The 150-kD antigen, antibodies to which were present in only 4% of patients with vitiligo, was expressed in approximately half of melanoma and control cells. These observations indicate that pigment cell antibodies in patients with vitiligo are directed most often to common tissue antigens expressed by pigment cells, but in approximately one-third of patients they are directed to antigens preferentially expressed on pigment cells.

It is known that different culture conditions may alter the expression of surface molecules. In particular, PMA, a melanocyte proliferation agent and a component of the medium used to grow melanocytes, can alter the expression of some melanocyte antigens. However, the finding that the antigens defined by vitiligo antibodies are expressed similarly on melanocytes grown both with and without PMA excludes the possibility that they are induced by the PMA in growth medium. In addition, these antigens were all expressed by some melanoma cells grown in different media, confirming that their expression is not an artifact induced by either PMA or other components of the medium used to grow melanocytes. Lastly, as antibodies to these antigens are naturally present in patients with vitiligo who have not been exposed to PMA or cultured melanocytes, the antigens that triggered their elicitation is clearly not a tissue culture artifact.

Our finding that antibodies to pigment cell antigens were present in 78% of 23 patients with vitiligo but in only 14% of 22 control individuals confirms our previous findings that they are more common in patients with vitiligo than in normal individuals [1–4,7,8]. These antibodies were most commonly directed to the 40–45-kD antigen, antibodies to which were present in 74% of patients, than to the 75-kD and 90-kD antigens, antibodies to which were present in 57% and 35% of patients, respectively. Only one patient (4%) had antibody response to the 150-kD or to the 35-kD antigens.

Some of these antigens were preferentially expressed on pigment cells and others appeared to be common tissue antigens. The most interesting antigen was the 90-kD antigen, antibodies to which were present in 35% of patients with vitiligo and in none of the control individuals. This antigen was selectively expressed on human pigment cells. It was expressed on 4 of 6 human pigment lines, but on none of 2 xenogeneic melanoma cell lines. By contrast, it was expressed on only 1 of 6 other control cell lines. The 35-kD antigen was expressed by only one of the human pigment cells and on none of the controls; however, antibody to it was present in only 4% of the patients with vitiligo and in none of the controls. The 75-kD and 40–45-kD antigens, antibodies to which were present in 57% and 74% of patients with vitiligo and 9% and 14% of control individuals, respectively, were common tissue antigens as they were expressed on most pigment and control cells. It is of interest that the pigment cell antibodies present in some normal individuals were directed to these two antigens. The 150-kD antigen, antibodies to which were present in only 4% of patients with vitiligo, was expressed in approximately half of melanoma and control cells. These observations indicate that pigment cell antibodies in patients with vitiligo are directed most often to common tissue antigens expressed by pigment cells, but in approximately one-third of patients they are directed to antigens preferentially expressed on pigment cells.

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Figure 2. Expression of antigens defined by vitiligo antibodies on a panel of pigment and control cell lines as measured by SDS-PAGE analysis and autoradiography of specific immunoprecipitates. Cells studied are human melanocyte (Mc); human melanoma (SK-mel-28, SK-mel-23, SK-mel-30, M20, and M14); murine melanoma (B16); hamster melanoma (HM54); erythroblasts (K562); colon carcinoma (SK-CO-1); lung carcinoma (A549); rhabdomyosarcoma (RD); baby hamster kidney (BHK-1), and normal human lymphocyte (NHL).
The role of the pigment cell antibodies in the pathogenesis of vitiligo is not known [11]. However, the facts that the antibodies are much more common in individuals suffering from vitiligo than in normal individuals, that there is a correlation between the presence and level of these antibodies and the extent [6] and activity of vitiligo [7], and that these antibodies can kill human melanocytes in vitro [8,9] suggest that they are involved in the pathogenesis of the disease. The present study indicates that these antibodies are directed to multiple antigens on the surface of pigment cells. The observation that some of these antigens are preferentially expressed on pigment cells whereas others appear to be common cellular antigens suggests that at least two distinct immune mechanisms could mediate the selective destruction of pigment cells, which is characteristic of vitiligo. One mechanism would be an immune response directed to antigens preferentially expressed on the surface of pigment cells, such as the 90-kD and 35-kD antigens. The other would be an immune response directed to common tissue antigens present on pigment cells, which selectively damages the pigment cells because they are more sensitive to immune injury. The latter possibility is supported by the report of Norris et al [8,9] that melanocytes are much more sensitive to toxic- or immune-mediated injury than keratinocytes or fibroblasts.

Lastly, our finding that antigens defined by antibodies in patients with vitiligo are also expressed on the surface of melanoma cells may explain the associations between melanoma and vitiligo [12,13].

REFERENCES