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Expression of Vitiligo Antigen on a Revertant Line of Hamster Melanoma Cells

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Our laboratory has recently reported that over 80% of patients with common vitiligo have circulating antibodies to cell-surface antigens on normal human melanocytes. The slow growth rate of these cells limits the assays that can be performed for antibody detection. We now have found that the antigens defined by vitiligo sera on melanocytes are also expressed on FF cells, a revertant line of hamster melanoma cells. These antigens can be detected both by indirect immunofluorescence and specific immunoprecipitation assays. The presence of "vitiligo" antigens on hamster FF cells will aid further study of the abnormal immune response in vitiligo.

Progress in understanding the pathogenesis of vitiligo has been limited by difficulties in studying this disease in vitro. Recently, normal human melanocytes have been grown in cell culture [1] making it possible to develop sensitive and quantitative assays of the immune response to pigmented cells in vitiligo. Using these cells, we have demonstrated that most individuals [2,3], as well as animals [4], with this disease have antibodies to pigment cells. The antibodies are directed to several cell-surface antigens, "vitiligo antigens," with M_r of 75K, 85K, and 240–250K which are selectively expressed by pigment cells. These findings indicate that vitiligo is associated with an immune response to pigment cell antigens and suggest that it is an autoimmune disease. However, continued studies of immune abnormalities in vitiligo are limited by the fastidiousness of melanocytes and the difficulties in maintaining them in culture.

We now report that the established hamster cell line, the FF, expresses the vitiligo antigens. The FF line is a revertant cell line of hamster melanocytic origin which has regained the capacity for density-dependent growth [5,6]. The presence of the vitiligo antigens on FF cells provides an additional cell line that will facilitate studies of immune abnormalities in vitiligo.

MATERIALS AND METHODS

Sera

Studies were conducted with sera from 15 persons with vitiligo and 12 normal individuals. All vitiligo sera were previously shown to have antibodies to surface antigens on melanocytes [3].

Cells

FF and RPMI (Roswell Park Memorial Institute) 1846 cells were maintained in Falcon T30 flasks in RPMI 1640 medium containing 10% fetal calf serum (GIBCO, Grand Island, New York), 2 U/ml penicillin (GIBCO), 2 μg/ml streptomycin (GIBCO), 10 mg/ml gentamycin (Irvine Scientific, Santa Ana, California), and 0.5 μg/ml fungizone (Irvine Scientific). Medium was changed biweekly, and cells subcultured every 2 weeks. Human melanocytes were maintained in culture as previously described [1].

FF is an aneuploid, contact inhibited hamster cell line. It was derived from the hamster amelanotic malignant melanoma cell line, RPMI 1846, during experimental studies of reverse transformation by nucleic acids from benign blue nevi of hamsters [5,6].

Radioiodination

Cell-surface macromolecules were radioiodinated by the lactoperoxidase technique as previously described [2]. Cell viability following iodination was over 90–95% by trypan blue exclusion. Labeled cells were thoroughly washed and solubilized in 0.5% NP-40. Particulate matter was removed by centrifugation at 12,000 $g \times 20$ min.

Assays

Radioactivity associated with labeled macromolecules was measured after precipitation with 10% trichloroacetic acid [7].

Antibodies to labeled surface antigens were assayed by immunoprecipitation with protein A-sepharose [2]. Briefly, 0.1 ml of a 1:10 dilution of serum was incubated with 0.1 ml of radioiodinated cell lysate at 4°C for 12 h, and bound antigens were immunoprecipitated by incubating with protein A-sepharose at 4°C for 2 h. All assays were performed in duplicate and the average value was recorded. Variation among replicate samples was less than 0.8%. There was a linear relation between the dilution of vitiligo serum used in the assay and specifically bound radioactivity, suggesting that the assay provides a quantitative measure of antibodies to melanocyte-associated antigens [3]. Using our established criteria for vitiligo antibodies (precipitation of 2.5% or more of acid-insoluble radioactivity), all patients with vitiligo had antibodies to melanocytes.

Polyacrylamide Gel Electrophoresis (PAGE)

Immunoprecipitates generated with vitiligo and normal sera were examined by sodium dodecyl sulfate (SDS)-8% PAGE under reducing conditions and radioautography as previously described [8].

Immunofluorescence Assays

Cells were plated in wells of microtiter plates (Lab-Tek Div., Miles Laboratories, Inc., Naperville, Illinois) at a density of 5×10^3 cells/well in 0.5 ml of culture media, and incubated at 37°C for 24 h. The cells were washed 3 times with 0.7 ml of Hanks' buffered salt solution per well, incubated with 0.5 ml of test serum diluted 1:10 in phosphate-buffered saline (PBS) for 30 min at 37°C, washed 3 times with 0.7 ml of PBS and reincubated for 30 min with fluorescein-conjugated goat antihuman IgG (Hyland Diagnostics, Deerfield, Illinois) diluted 1:40 in PBS. The cells were washed again 3 times with PBS and examined immediately with a Nikon binocular microscope (Nikon Inc., Garden City, New York) equipped to detect immunofluorescence.

RESULTS

The results obtained when vitiligo and normal human sera were tested for reactivity against normal human melanocytes and hamster FF and RPMI 1846 cells by immunoprecipitation are summarized in Table I. As previously shown, vitiligo, but not normal sera, reacted strongly with radioiodinated surface antigens on normal human melanocytes. Strong reactivity was also observed between vitiligo, but not normal, sera and the FF cells. These differences were highly significant ($p < 0.0005$). By contrast, vitiligo sera reacted poorly, and to no greater

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Abbreviations:
CIF: contact inhibitory factor
FF: a revertant cell line of hamster melanocytic origin
PAGE: polyacrylamide gel electrophoresis
PBS: phosphate-buffered saline
SDS: sodium dodecyl sulfate

TABLE I. *Reactivity of vitiligo antibodies with normal, revertant, and malignant melanocytic cells*

Diagnosis	Sera no.	% Acid-insoluble radioactivity immunoprecipitated[a]		
		Normal human melanocytes	FF cells	RPMI 1846
Vitiligo	1	4.1	3.8	1.2
	2	9.8	6.1	1.7
	3	3.8	2.9	0
	4	5.2	4.4	2.0
	5	7.3	6.7	1.9
	6	6.4	5.7	2.1
	7	7.6	6.8	0.9
	8	5.8	4.4	1.1
	9	4.9	3.7	0.7
	10	8.6	7.1	1.8
	11	4.7	3.2	1.1
	12	5.4	3.1	1.1
	13	3.9	2.8	0.7
	14	4.3	2.9	0.3
	15	4.7	3.6	0.9
	Average	5.77 ± 1.82(A)	4.48 ± 1.57(B)	1.17 ± 0.63(C)
Normal	1	1.9	2.0	1.7
	2	0.7	1.7	1.4
	3	0.4	1.6	0.6
	4	1.1	1.4	1.5
	5	1.4	1.4	1.7
	6	1.7	1.8	0.2
	7	0.5	1.6	0.4
	8	0.8	0.9	1.1
	9	0.9	1.7	0.4
	10	1.2	1.0	1.0
	Average	1.06 ± 0.49(D)	1.51 ± 0.35(E)	1.0 ± 0.57(F)

A vs D ($p < 0.0005$); B vs E ($p < 0.005$); C vs F ($p > 0.25$); A vs C ($p < 0.0005$); B vs C ($p < 0.0005$).

[a] Calculated from:

$$\frac{\text{(cpm immunoprecipitated by serum)}}{\text{acid-insoluble cpm in } ^{125}\text{I-labeled cell lysates}} \times 100$$

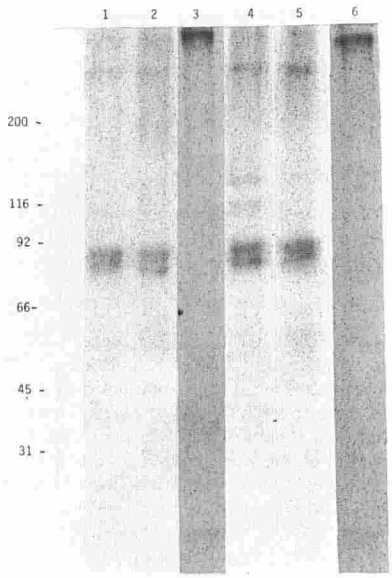

FIG 1. Radioautograph of SDS-PAGE profile of detergent-soluble, radioiodinated surface macromolecules of normal human melanocytes (*lanes 1, 2, 3*) and FF cells (*lanes 4, 5, 6*) immunoprecipitated by sera of patients with vitiligo (lanes 1, 2, 4, 5) and normal individuals (*lanes 3, 6*).

extent than normal sera, with RPMI 1846 cells. The vitiligo sera used in this study had previously been shown to be nonreactive to a number of other control cells including hepatoma, colon carcinoma, fibroblasts, etc. [9]. These results indicate that FF cells are useful as target cells for assay of vitiligo antibodies.

The surface antigens reacting with vitiligo antibodies on FF cells were identified by SDS-PAGE analysis of specific immunoprecipitates generated in a similar manner from detergent lysates of radioiodinated normal human melanocytes and FF cells. The results are illustrated in Fig 1. Vitiligo sera immunoprecipitated 3 radioiodinated antigens from both melanocytes and FF cells. On the basis of their migration through polyacrylamide the antigens defined on both cell lines were similar, with M_r of 75K, 85K, and 240–250K. None of these antigens was immunoprecipitated by normal human sera.

Indirect immunofluorescence testing showed that vitiligo sera reacted to human melanocytes and FF cells, giving a granular pattern of fluorescence. With appropriate focusing the staining could be seen to involve the cell surface (Fig 2*a*). Seventy percent or more of the FF cells reacted with vitiligo sera whereas only 5% or less of the RPMI 1846 cells did so (Fig 2*b*). No positive granular staining was noted with the 12 normal human control sera against human melanocytes, FF cells, or RPMI 1846.

DISCUSSION

The results of this study indicate that hamster FF cells express vitiligo antigens. These antigens are defined by sera of

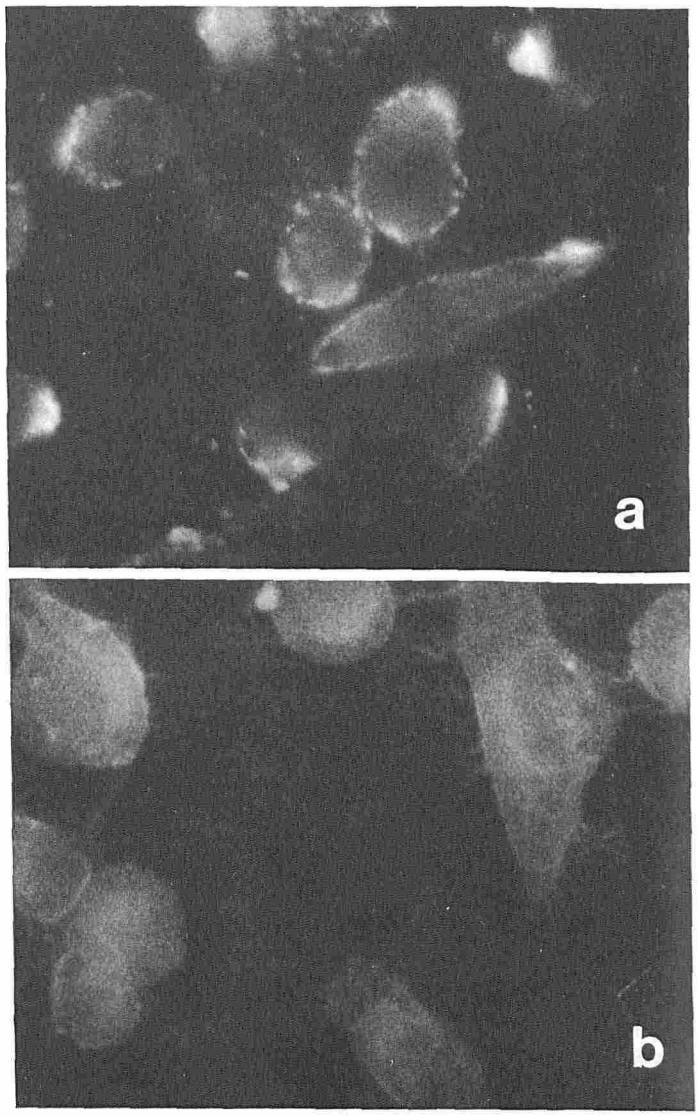

FIG 2. *a*, Immunofluorescence photomicrograph of unfixed FF cells reacted with vitiligo serum. A positive reaction is seen as bright granular fluorescence on the surface of the cells. Original magnification × 400. *b*, indirect immunofluorescence of unfixed RPMI 1846 cells reacted with vitiligo serum. Bright fluorescence is absent.

patients with vitiligo and can be detected by both indirect immunofluorescence and specific immunoprecipitation assays. Both the amount of vitiligo antigen expressed by these cells (as determined from the amount of radioactivity immunoprecipitated by vitiligo sera) and their nature (as defined by migration on SDS-PAGE) are similar to the amount and type of vitiligo antigen expressed by normal human melanocytes. Migration patterns on SDS-PAGE indicate that the antigens precipitated by vitiligo sera have M_r of 75K, 85K, and 240–250K. The cellular location and migration pattern of these antigens is identical to those seen on normal human melanocytes.

Indirect immunofluorescence testing confirmed that the immunoprecipitation assay was measuring antibodies directed to antigens on the FF cell line. Vitiligo sera reacted strongly to FF cells but not to the RPMI 1846 cells. A correlation was seen between the intensity of granular staining and the binding activity of the sera as found by specific immunoprecipitation.

These findings indicate that FF cells provide a suitable cellular substrate for studies of immune abnormalities in vitiligo. The observation that the vitiligo antigens are present on the revertant FF cell line but not on the malignant RPMI 1846 cells is interesting for several reasons. It demonstrates that a phenotypically revertant melanocytic cell line has reexpressed several differentiation antigens absent in the malignant parental line. It also shows that these antigens are common to melanocytes of both hamster and human origins, since hamster cells react with antibodies generated in human vitiligo patients. The hamster FF line bearing these differentiation antigens has previously been shown to produce a diffusible contact inhibitory factor (CIF) which restores the capacity for density-dependent growth to malignant melanocytes of hamster [10], murine [11], and human [11] origins. Therefore it is fascinating to find in our most recent studies that CIF also *induces* the expression of all 3 vitiligo antigens on malignant melanoma cells of both human and mouse.* Further investigation of the appearance of these antigens will help in the evaluation of the role of these cell-surface proteins in the pathogenesis of vitiligo.

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