PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE **181**, 423–426 (1986)

Antibodies to Surface Antigens of Pigmented Cells in Animals with Vitiligo[1] (42275)

GAIL K. NAUGHTON,* MARY MAHAFFEY,† AND JEAN-CLAUDE BYSTRYN*,[2]

*Department of Dermatology, New York University School of Medicine, New York, New York 10016, and
†Department of Anatomy and Radiology, College of Veterinary Medicine,
University of Georgia, Athens, Georgia 30603

Abstract. All of 24 animals (dogs, cats, and horses) with vitiligo were found to have antibodies to pigmented cells that could be detected by specific immunoprecipitation of radioiodinated, detergent-soluble surface macromolecules, and by indirect immunofluorescence on viable cells. These antibodies were not detected in 17 normal animals of the same species. The antibodies were directed to an 85-kDa surface antigen selectively expressed by pigmented cells that was not present on nonpigmented control cells. These observations suggest that vitiligo in animals is an autoimmune disease mediated to pigmented cells.

Vitiligo is a depigmentary skin disease manifested by selective destruction of melanocytes and subsequent loss of pigmentation. The disease occurs in man as well as in a variety of animals, including Lippizzaner and Arabian horses (1), Belgian Tervuren dogs (2), Siamese cats (3), Sinclair swine (4), and Dam chickens (5). Although the cause of vitiligo is not known, our laboratory has recently shown that the majority of patients with vitiligo have circulating antibodies to antigens, "vitiligo antigens," selectively expressed by normal human melanocytes (6) and pigmented melanoma cells (7) suggesting an autoimmune involvement. These antigens are surface proteins with approximate mol wt of 75, 85, and 250KDa (7). Several observations suggest involvement of immune mechanisms in the pathogenesis of animal vitiligo as well. The appearance of vitiligo in Dam chickens is delayed by neonatal bursectomy (5), a procedure which impairs humoral immunity. In addition, vitiligo can be induced in chimpanzees immunized to melanoma cells (8), and horses (1) and swine (4) in whom melanoma regresses spontaneously develop vitiligo, suggesting that immune reactions to malignant pigmented cells may destroy normal pigmented cells. However, there has been no direct demonstration of an alteration in specific immunity to pigmented cells in animals with vitiligo.

In this study we have used immunoprecipitation and immunofluorescence assays to examine the antibody response to pigmented cells of animals with vitiligo.

Materials and Methods. *Sera.* Sera were collected from breeds of dogs, cats, and horses who have a tendency to develop vitiligo. Sera from 28 Belgian Tervuren dogs (17 with vitiligo), 7 Siamese cats (4 with vitiligo), and 6 Arabian horses (3 with vitiligo) were collected, aliquoted into 1-ml fractions, and stored at −70°C until used.

Cells. Two lines of human pigmented melanoma cells, SK-Mel 19 and SK-Mel 23, were kindly provided by Dr. Jorgen Fogh (Memorial Sloan–Kettering, Rye, N.Y.). These cells have previously been shown to express vitiligo antigens (7). Control cells included two lines of nonpigmented human melanoma cells (HM31 and HM49) established in our laboratory, as well as normal human fibroblasts, neuroblastoma, hepatoma, and colon carcinoma cells.

Immunoprecipitation assay for pigment cell antibodies. Cell-surface macromolecules on melanoma and control cells were radioiodinated by the lactoperoxidase method and solubilized with 0.5% NP-40 (10). Immunoprecipitation with protein A–sepharose (Pharmacia Inc., Piscataway, N.J.) was performed as previously described (6, 9). All assays were

[1] This work was supported in part from NIH Research Grant AM 27663-04, and a grant from the Skin Cancer Foundation, New York, N.Y.

[2] To whom reprint requests should be addressed: Department of Dermatology, NYU School of Medicine, 550 First Avenue, New York, N.Y. 10016.

0037-9727/86 $1.50

performed in duplicate, and the average value used. Specific binding was calculated by substracting from the cpm bound by test serum the cpm bound by an equal volume of normal serum. Sera were considered positive for antibodies when they bound specifically 2.5% or more of the acid-insoluble radioactivity (9) in cell lysates.

Immunofluorescence assays for pigment cell antibodies. Human pigmented melanoma and control cells were plated in microtiter plates (Lab–Tek Div., Miles Laboratories, Inc., Naperville, Ill.) at a density of 4×10^3 cells/well in 0.4 ml of appropriate culture media. After 24 hr the cells were washed thoroughly with Hanks' balanced salt solution (HBSS) and incubated with 0.5 ml of animal serum diluted 1:10 in phosphate-buffered solution (PBS). Following a 30-min incubation at 37°C the cells were washed with PBS and reincubated with fluorescein-conjugated goat anti-dog, cat, or horse IgG (Hyland Diagnostics, Deerfield, Ill.) diluted 1:60 in PBS. After a 30-min incubation the cells were washed extensively and examined with a Nikon binocular microscope (Nikon Inc., Garden City, N.Y.) equipped for immunofluorescence.

Polyacrylamide gel electrophoresis (PAGE). Immunoprecipitates generated with vitiligo and normal animal sera were examined by sodium dodecyl sulfate (SDS)–8% PAGE under reducing conditions and radioautography as previously described (6, 10).

Results. Sera of 24 animals with and 17 without vitiligo were tested for antibodies to pigmented cells by specific immunoprecipitation. The results are summarized in Table I. In every instance sera of animals with vitiligo bound much more of the radioactivity associated with detergent-soluble, labeled, surface macromolecules of pigmented cells than did sera of similar animals without vitiligo. Seventeen Tervuren dogs, four Siamese cats, and three Arabian horses with spontaneous vitiligo bound specifically on the average 8.26 ± 4.7%, SD 1, 8.1 ± 2.9%, and 11.2 ± 2.9%, respectively, of the radioactivity associated with acid-insoluble macromolecules in detergent lysates of radioiodinated pigmented melanoma cells. By contrast, animals of the same species without vitiligo bound specifically only 1.28 ± 1.02%, 0.3 ± 0.3%, and 0.9 ± 0.5%, respectively, of this radioactivity. These differences are significant at the $P < 0.05$ level using the Student t test (11). Based on the ability to bind specifically at least 2.5% of the radioactivity

TABLE I. IMMUNOPRECIPITATION OF RADIOIODINATED SURFACE MACROMOLECULES OF PIGMENTED MELANOMA CELLS BY SERA OF ANIMALS WITH AND WITHOUT VITILIGO

Species	Diagnosis	Animal No.	% Acid-insoluble radio-activity immuno-precipitated[a]	Average
Dog	Vitiligo	1	8.0	8.29 ± 2.1%
		2	11.2	
		3	13.0	
		4	9.6	
		5	7.1	
		6	8.4	
		7	6.9	
		8	11.0	
		9	7.7	
		10	7.3	
		11	8.0	
		12	7.3	
		13	13.4	
		14	9.1	
		15	3.8	
		16	5.1	
		17	4.1	
	Normal	18	2.2	1.28 ± 1.02
		19	2.3	
		20	0.9	
		21	2.1	
		22	1.4	
		23	0.7	
		24	1.4	
		25	1.2	
		26	0.5	
		27	1.1	
		28	0.3	
Cat	Vitiligo	1	10.9	8.1 ± 2.9
		2	6.9	
		3	6.4	
		4	8.2	
	Normal	5	0.9	0.3
		6	0	
		7	0	
Horse	Vitiligo	1	11.3	11.2 ± 2.9
		2	10.9	
		3	8.3	
	Normal	4	0.3	0.9 ± 0.9
		5	0.9	
		6	1.4	

[a] % Acid-insoluble radioactivity immunoprecipitated. Calculated from (cpm immunoprecipitated by serum/acid-insoluble cpm in cell lysate) × 100 ± SD.

TABLE II. ANTIBODIES TO PIGMENTED CELLS IN ANIMALS WITH VITILIGO

Species	Diagnosis	No. studied	% With antibodies[a]
DOG	Vitiligo	17	100
	Normal	11	0
CAT	Vitiligo	4	100
	Normal	3	0
HORSE	Vitiligo	3	100
	Normal	3	0

[a] Sera binding at least 2.5% of acid-insoluble cpm in detergent lysates of ^{125}I-labeled pigmented mean melanoma cells (SK-23).

associated with acid-insoluble macromolecules, all of the animals with vitiligo, but none of those without, had antibodies to pigmented cells (Table II).

These results were confirmed by indirect immunofluorescence studies on viable cells. Granular staining of surface antigens was seen when the A panel of sera of four dogs, two cats, and two horses with vitiligo were reacted with pigmented melanomas. There was no staining when the same sera were reacted with nonpigmented cells, and sera of eight normal animals did not stain any of the cells.

To examine the specificity of the pigment cells antibodies found in animals with vitiligo, three antibody-positive sera from each species were reacted with a panel of pigmented and nonpigmented cells (Table III). All sera reacted strongly with pigmented melanoma cells (SK-Mel 19 and 23) but weakly or not at all with nonpigmented melanomas (HM31 and HM49) or control cells (normal fibroblasts, neuroblastoma, hepatoma, colon carcinoma). Normal animal sera did not react with any of the cells.

The antigen defined by antibodies in animals with vitiligo was identified by SDS–PAGE and radioautography of specific immunoprecipitates generated from lysates of radioiodinated pigmented melanoma cells. All vitiligo sera from dogs, cats, and horses immunoprecipitated a single labeled protein with mol wt of approximately 85K. Representative gels are illustrated in Fig. 1. This protein was not precipitated by normal sera or by vitiligo sera reacted with the control cells listed in Table III.

Discussion. The most important finding of this study is that all the animals we studied with vitiligo had circulating antibodies to a surface antigen of pigmented cells. These antibodies were not found in animals without vitiligo. The antibodies were directed to surface antigen with mol wt of approximately 85K which was selectively expressed on pigmented melanoma cells. This antigen was strongly expressed on pigmented melanoma cells, but was not detectable on nonpigmented melanoma cells or a variety of other control cells. The antigen is not species-specific, since it could be identified on human cells using animal sera and different species of animals (dogs, cats, horses) with vitiligo had antibodies to a similar antigen. This antigen is similar in its migration on SDS–PAGE, its location on the external surface of the cell membrane, and its selective expression on pigmented cells to one of a trio of vitiligo antigens defined on the surface of normal human melanocytes and pigmented melanoma cells by antibodies in persons with vitiligo (6, 7, 9). Thus, both animals and humans with vitiligo appear to have a similar abnormal antibody response to pigmented cells.

This is the first demonstration of an abnormality in specific immune response to pig-

TABLE III. SPECIFICITY OF ANTIBODIES TO PIGMENTED CELLS IN ANIMALS WITH VITILIGO

Cells[a]	% Acid-insoluble radioactivity immunoprecipitated by sera of[b] Dog	Cat	Horse
Pigmented melanoma			
SK 19	9.3	6.2	8.3
SK 23	9.0	8.1	11.2
Nonpigmented melanoma			
HM 49	2.2	1.4	1.9
HM 31	1.8	1.3	1.4
Other nonpigmented			
Fibroblasts	1.8	1.1	0.8
Neuroblastoma	1.3	1.4	1.5
Hepatoma	0.5	0.8	1.2
Colon carcinoma	1.2	1.1	1.7

[a] Detergent lysate of ^{125}I-labeled cells.

[b] Average binding by sera of three animals of the same species.

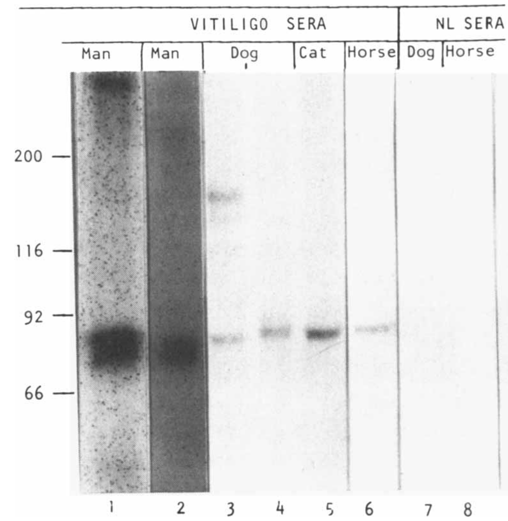

FIG. 1. SDS–PAGE profile of radioiodinated, detergent-soluble, surface macromolecules of pigmented human melanoma cells SK-MEL 23 immunoprecipitated by sera of persons (lanes 1 and 2), dogs (lanes 3 and 4), cat (lane 5), and horses (lane 6) with vitiligo; and with normal dog (lane 7) and horse (lane 8) sera. Sera of animals with vitiligo immunoprecipitated a labeled protein with mol wt of approximately 85 kDa. Persons with vitiligo immunoprecipitated two additional proteins with mol wts of approximately 75 and 250 kDa.

mented cells in animals with vitiligo. The finding supports, but does not prove, the concept that vitiligo is an autoimmune disease mediated by antibodies to pigmented cells and suggests that the pathogenesis of this disease in man and animals is similar.

1. Lerner AB, Cage GW. Melanomas in horses. Yale J Biol Med **46**:646, 1973.
2. Mahaffey MD, Yarbrough KM, Munnell F. Focal loss of pigment in the Belgian Tervuren dog. J Amer Vet Med Assoc **173**:390.J, 1978.
3. Lerner AB, Nordlund JJ. Vitiligo: The loss of pigment in skin, hair and eyes. J Dermatol **5**:1, 1978.
4. Millikan LE, Hook RR, Manning PJ. Gross and ultrastructural studies in a new melanoma model: The Sinclair swine. Yale J Biol Med **46**:631, 1973.
5. Smyth JR, Boissy RE, Fite KV. The DAM chicken: A model for spontaneous postnatal cutaneous and ocular amelanosis. J Hered **72**:150, 1981.
6. Naughton GK, Eisinger M, Bystryn JC. Antibodies to melanocytes in vitiligo. J Exp Med **158**:246, 1983.
7. Naughton GK, Eisinger M, Bystryn JC. Identification and partial characterization of vitiligo antigens. Clin Res **32**:605A, 1984.
8. Hornung MA, Krementz ET. Specific tissue and tumor responses of chimpanzees following immunization against human melanoma. Surgery (St. Louis) **75**:477, 1974.
9. Naughton GK, Eisinger M, Bystryn JC. Detection of antibodies to melanocytes in vitiligo by specific immunoprecipitation. J Invest Dermatol **81**:540, 1983.
10. Heaney-Kieras J, Bystryn JC. Identification and purification of a 75 k dalton cell-surface human melanoma associated antigen. Cancer Res **42**:2310, 1982.
11. Bartolucci AA, Fraser MD. Comparative step-up and composite tests for selecting prognostic indicators associated with survival. Biom J **19**:437, 1977.

Received July 2, 1985. P.S.E.B.M. 1986, Vol. 181.
Accepted November 18, 1985.