

CELL SURFACE ANTIGENS OF HUMAN  
MALIGNANT MELANOMA

III. Recognition of Autoantibodies with  
Unusual Characteristics\*

By HIROSHI SHIKU, TOSHITADA TAKAHASHI, LOIS A RESNICK,  
HERBERT F OETTGEN, AND LLOYD J OLD

(From the Memorial Sloan-Kettering Cancer Center, New York 10021)

Our serological analysis of malignant melanoma has focused on cell surface antigens detected by reactions with autologous serum (1, 2). Cultured lines of melanoma cells and companion skin fibroblasts from the same individual serve as standard target cells in mixed hemadsorption (MHA) and immune adherence (IA) assays. Absorption tests with cells from autologous, allogeneic, and xenogeneic sources determine the specificity of reactions observed between melanoma cells and autologous serum. In this way, three distinctive melanoma surface antigens, designated AU, BD, and AH, have been defined (1, 2). AU and BD belong to a class of individually distinct surface antigens which show an absolute restriction to autologous melanoma cells (1, 2). The other, AH, represents a class of shared melanoma antigens which has been detected on 5 out of 12 melanomas, but not on corresponding autologous fibroblasts or on other normal or malignant tissues (2)

This report summarizes the analysis of three additional autologous serum reactions with melanoma cells. In the two instances where autologous fibroblast lines were available as target cells, these did not react in direct tests with autologous serum, thus giving the impression of melanoma specificity. However, absorption tests indicated that the antigens being detected occur on a range of other cell types, including fibroblasts, with a pattern of antigen distribution that is unique in our experience.

Materials and Methods

*Tissue Culture.* For derivation of melanoma and other cell lines, see our previous reports (1, 2). Tissue culture lines are maintained in Eagle's minimum essential medium supplemented with 2 mM glutamine, 1% nonessential amino acids, penicillin 100 IU/ml, streptomycin 100  $\mu$ g/ml, and 10-20% fetal bovine serum.

*Serology* IA and MHA assays were performed as described previously (1, 2) Titer refers to the highest dilution giving 10% positive cells in IA assays and 5% in MHA assays For absorption analysis (1, 2), cultured cells were harvested by mechanical scraping or by 0.05% EDTA, preliminary tests showed that the antigens being detected were not destroyed by EDTA at this concentration After removal of the absorbing cells by centrifugation, the serum was titrated for residual reactivity on autologous melanoma cells.

\* This work was supported in part by grants from The Cancer Research Institute, Inc., The Oliver S. and Jennie R. Donaldson Charitable Trust, and The National Cancer Institute (PO1 CA-19267 and CA-08748)

## Results

*Direct IA and MHA Assays with Autologous Melanoma Cells and Normal Skin Fibroblasts.* Sera from three patients showing reactivity with cultured autologous melanoma cells were selected from our original survey (1, 2). Initial study indicated that IA was optimal for demonstrating reactions with AV and AN sera and MHA best for AT serum. AV and AT sera reacted most strongly at 24°C, whereas AN serum showed optimal reactivity at 4°C. The titers of different serum specimens from these patients ranged from 1/4–1/16 for AV, 1/40–1/320 for AN, and 1/4–1/64 for AT sera. Cultures of skin fibroblasts were available from patients AV and AT. Serum that was positive with autologous melanoma cells did not react with autologous fibroblasts.

### *Absorption Analysis*

**AV SERUM.** Fig. 1a illustrates a single absorption test with the AV serum specimen selected for analysis, and Table I summarizes the results of a series of absorption tests with this serum. In contrast to negative direct tests with autologous fibroblasts, the reactivity of AV serum with autologous melanoma was absorbed by autologous fibroblasts as well as autologous melanoma cells. Autologous hematopoietic cells, however, failed to absorb reactivity, indicating that the antigen (or antigens) detected by this absorption system is present only on certain differentiated cell types. AV antigen also has characteristics of an alloantigen; some allogeneic melanoma cells (e.g., SK-MEL-27 and SK-MEL-28) and companion fibroblasts absorbed AV reactivity, whereas comparable cells from other individuals (e.g., SK-MEL-13 and SK-MEL-37) did not. The antigen occurs on the surface of xenogeneic cells as well; nucleated cells from monkey, chicken, and mouse absorbed reactivity from AV serum. Autologous, allogeneic, and xenogeneic erythrocytes lacked AV antigen.

**AN SERUM.** Fig. 1b and Table II represent absorption analysis of the selected AN serum. A wide variety of cultured and noncultured nucleated cells absorbed AN reactivity. Unlike the AV system, the AN antigen is present on lymphoid cells and granulocytes. However, like AV, it has characteristics of an alloantigen and also of a differentiation antigen (present on some tissues but not on others from the same individual). This is illustrated by absorption with various cell types from patient BD; melanoma cells (SK-MEL-37), companion fibroblasts, and platelets did not absorb AN reactivity, but lymphoid cells and granulocytes did. AN antigen is found also on some xenogeneic cells such as cultured monkey kidney, but not on mouse or chicken fibroblasts.

**AT SERUM.** As shown in Fig. 1c and Table III, the antigen defined by MHA reactions of the selected AT serum on autologous melanoma has been found on all cultured and noncultured nucleated cell populations tested, autologous, allogeneic, or xenogeneic. Erythrocytes are the only cell type lacking AT antigen.

## Discussion

The choice of autologous target cells to search for antibody to surface antigens of melanoma cells was intended to limit the range of antibodies that could be detected, excluding therefore the contribution of alloantibodies present natu-

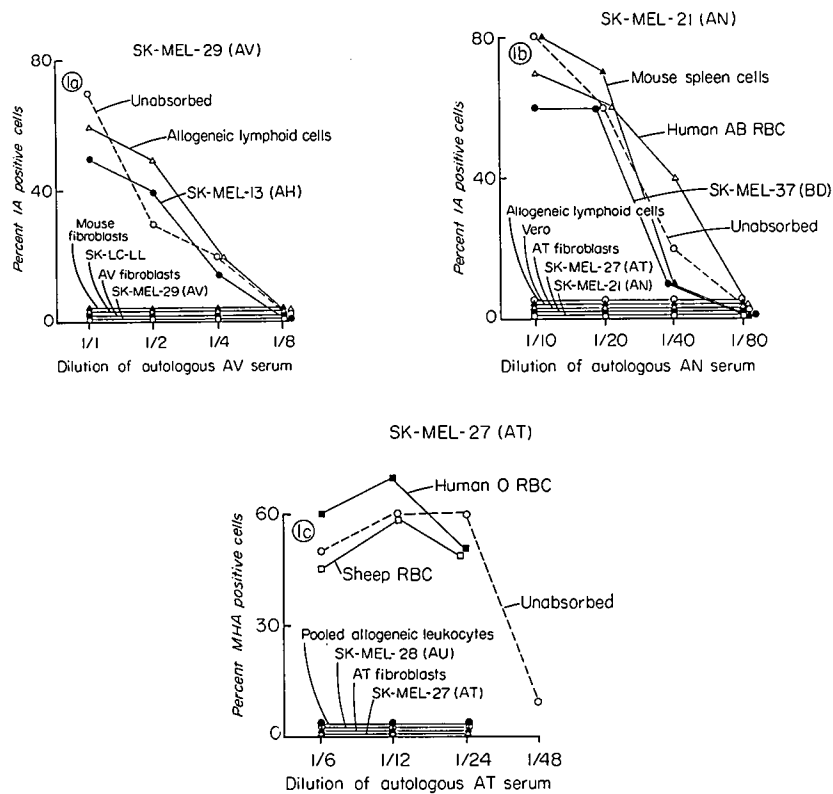


FIG 1. Examples of individual absorption tests with AV, AN, and AT sera. (a) Autologous melanoma and fibroblasts, allogeneic lung cancer, and xenogeneic mouse fibroblasts absorbed IA reactivity of AV serum to autologous melanoma SK-MEL-29. Allogeneic melanoma SK-MEL-13 and allogeneic lymphoid cells did not (b) Autologous melanoma, allogeneic melanoma SK-MEL-27 and its companion fibroblast line, allogeneic lymphoid cells, and xenogeneic monkey kidney cells absorbed IA reactivity of AN serum to autologous melanoma SK-MEL-21. Allogeneic melanoma (SK-MEL-37), type AB erythrocytes, and xenogeneic mouse spleen cells did not. (c) All nucleated cells tested absorbed MHA reactivity of AT serum to autologous melanoma SK-MEL-27. Type O erythrocytes and xenogeneic sheep erythrocytes did not

TABLE I  
Absorption of IA Reactivity of AV Serum for Autologous Melanoma SK-MEL-29

Positive absorption		Negative absorption	
Autologous	Allogeneic	Autologous	Allogeneic
Cultured melanoma cells SK-MEL-29 (AV)	Cultured nonmelanoma cells SK-LMS-1 (leiomyosarcoma)	Normal cells Lymphoid cells	Cultured melanoma cells SK-MEL-13 (AH)
Cultured normal cells AV fibroblasts	SK-RC-4 (renal cell ca) T-24 (bladder ca)	Granulocytes Platelets	SK-MEL-37 (BD)
Allogeneic	ME 180 (cervical ca)	Erythrocytes	Cultured normal cells AH fibroblasts
Cultured melanoma cells SK-MEL-27 (AT)	SK-LC-LL (lung ca)	Allogeneic	BD fibroblasts
SK-MEL-28 (AU)	Xenogeneic	Normal cells and serum	Xenogeneic cells and serum
SK-MEL-30 (AW)	Cultured monkey kidney cells	Lymphoid cells	Sheep erythrocytes
MeWo (BD)	Cultured chicken fibroblasts	Granulocytes	Fetal bovine serum
Cultured normal cells AT fibroblasts AU fibroblasts	Cultured mouse fibroblasts Mouse spleen cells	Platelets Erythrocytes Serum	Mouse erythrocytes

TABLE II  
Absorption of IA Reactivity of AN Serum for Autologous Melanoma SK-MEL-21

Positive absorption		Negative absorption	
Autologous	Allogeneic	Allogeneic	Xenogeneic cells and serum
Cultured melanoma cells	Cultured nonmelanoma cells	Cultured melanoma cells	Sheep erythrocytes
SK-MEL-21 (AN)	SK-LMS-1 (leiomyosarcoma)	SK-MEL-37 (BD)	Fetal bovine serum
Allogeneic	Sal III (breast ca)	Cultured normal cells	Mouse spleen cells
Cultured melanoma cells	HEp-2 (larynx ca)	BD fibroblasts	Mouse erythrocytes
SK-MEL-13 (AH)	SK-LC-LL (lung ca)	Cultured nonmelanoma cells	Cultured mouse fibroblasts
SK-MEL-19 (AL)	WI-38 (fetal lung fibroblasts)	ME 180 (cervical ca)	Cultured chicken fibroblasts
SK-MEL-27 (AT)	PHEL-6 (fetal lung fibroblasts)	Normal cells and serum	
SK-MEL-28 (AU)	Normal cells	Platelets	
MeWo (BI)	Lymphoid cells (including BD)	Erythrocytes	
Cultured normal cells	Granulocytes (including BD)	Serum	
AH fibroblasts	Xenogeneic		
AT fibroblasts	Cultured monkey kidney cells		

TABLE III  
Absorption of MHA Reactivity of AT Serum for Autologous Melanoma SK-MEL-27

Positive absorption		Negative absorption	
Autologous	Allogeneic	Allogeneic	Autologous normal cells
Cultured melanoma cells	Cultured melanoma cells	Normal cells	Erythrocytes
SK-MEL-27 (AT)	SK-MEL-13 (AH)	Pooled leukocytes	Allogeneic normal cells
Cultured normal cells	SK-MEL-23 (AP)	Pooled tonsil leukocytes	Erythrocytes
AT fibroblasts	SK-MEL-28 (AU)	Xenogeneic	Xenogeneic cells and serum
Normal cells	SK-MEL-29 (AV)	Cultured monkey kidney cells	Sheep erythrocytes
Leukocytes	MeWo (BI)	Cultured chicken fibroblasts	Fetal bovine serum
Platelets	Cultured nonmelanoma cells	Cultured mouse fibroblasts	Mouse erythrocytes
Allogeneic	T-24 (bladder ca)	Mouse spleen cells	
Cultured normal cells			
AU fibroblasts			

rally or as a consequence of transfusion or pregnancy. When autologous reactions are observed, absorption analysis provides the means to test other autologous cells as well as allogeneic and xenogeneic cells for corresponding antigen.<sup>1</sup> Table IV summarizes the characteristics of the seven surface antigens which have now been recognized on melanoma cells through application of this serological approach, and for purposes of classification, three categories of melanoma surface antigens can be distinguished: (a) unique melanoma-specific antigens; restricted to autologous melanoma (e.g., AU, BI, and BD); (b) shared melanoma-specific antigens; found on some (but not all) allogeneic melanoma cells, but not on other normal or malignant tissues (e.g., AH); and (c) antigens not restricted to melanoma cells (e.g., AV, AN, and AT). The autoantibodies that detect this last category of surface antigens have a surprisingly complex pattern of reactivity as revealed by absorption tests. For instance, anti-AV shows autoreactivity (absorbed by autologous melanoma and fibroblasts), alloreactivity (absorbed by melanoma and corresponding fibroblasts from some individuals but not from others), and xenoreactivity (absorbed by monkey, mouse, and chicken tissues). In addition, the antigen (or antigens) detected by anti-AV shows the characteristics of a differentiation antigen, being present on

<sup>1</sup> For a consideration of typing of melanoma surface antigens by absorption tests, see reference 2.

TABLE IV  
*Cell Surface Antigens of Human Malignant Melanomas Defined by  
 Absorption Tests with Autologous Serum*

Antigen system	AU*	BI†	BD‡	AH§	AV	AN	AT
Serological test	MHA	MHA	IA	IA	IA	IA	MHA
Absorbed with							
Autologous cells							
Cultured melanoma cells	+	+	+	+	+	+	+
Cultured skin fibroblasts	-	-	-	-	+	-	+
Lymphoid cells	-	-	-	-	-	-	+
Erythrocytes	-	-	-	-	-	-	-
Allogeneic cells and serum							
Cultured melanoma cells	-	-	-	+ or -	+ or -	+ or -	+
Cultured skin fibroblasts	-	-	-	-	+ or -	+ or -	+
Cultured nonmelanoma cells	-	-	-	-	+	+ or -	+
Lymphoid cells	-	-	-	-	-	-	+
Erythrocytes	-	-	-	-	-	-	-
Serum	-	-	-	-	-	-	-
Xenogeneic cells and serum							
Cultured monkey kidney cells	-	-	-	-	+	+	+
Cultured chicken fibroblasts	-	-	-	-	+	-	+
Cultured mouse fibroblasts	-	-	-	-	+	-	+
Mouse spleen cells	-	-	-	-	+	-	+
Mouse erythrocytes	-	-	-	-	-	-	-
Sheep erythrocytes	-	-	-	-	-	-	-
Fetal bovine serum	-	-	-	-	-	-	-
Classification of antigen							
	Melanoma specific individual	Melanoma specific individual	Melanoma specific individual	Melanoma specific shared	Not mela- noma specific	Non mela- noma specific	Not mela- noma specific

some cell types but not on others from the same individual. Anti-AN has a similarly broad and complex reactivity, but absorption analysis shows that it detects different specificities than anti-AV. The antigen detected by anti-AT has the widest distribution of all. Nucleated cells from human, mouse, monkey, and chicken absorb anti-AT activity. Only erythrocytes appear to lack AT antigen.

What leads to the formation of these broadly reactive autoantibodies to cell surface components of nucleated cells, how frequently will they be detected in patients with melanoma and other disease states, and can they bring about tissue injury? Autoantibodies to various constituents of normal cells are known to occur with increased frequency in patients with cancer and are generally thought to arise as a consequence of antibody to tumor products that cross-react with normal tissues (3-6). In this regard, it may be of importance that the three melanoma patients with the broadly reactive antibodies (AV, AN, AT) had undergone chemotherapy, whereas patients AU, BD, and AH with melanoma-specific antibody had not. The possibility that chemotherapy with consequent tissue injury leads to an augmented or abnormal immune response to normal cellular components is now being directly investigated.

Recognition of this class of autoantibodies to cell surface components present on both normal and malignant cells adds yet another complication to the search for characteristic antigens of human cancer. For instance, several past studies have claimed tumor specificity on the basis of serological reactions with malignant cells but not with normal fibroblasts from the same individuals. We now

see that this sort of control is inadequate; AV and AT fibroblasts did not react with autologous serum in direct tests, but could remove reactivity for autologous melanoma cells in absorption tests. Nor can absorption with peripheral lymphocytes, a convenient source of normal cells, be depended upon to prove tumor specificity even in autologous systems; anti-AV's reactivity for autologous melanoma was not removed by autologous lymphocytes, but was absorbed by fibroblasts. As these autoantibodies may arise unexpectedly, it cannot be assumed that sequential serum specimens drawn from the same patient, even over a short period of time, will be detecting the same specificities. The possible presence of these broadly reactive antibodies to cell surface components will have to be taken into consideration when monitoring and interpreting changes in serological reactivity of patients to autologous cancer cells.

### Summary

The sera of three patients with malignant melanoma showing reactivity with surface antigens of cultured autologous melanoma cells were analyzed by mixed hemadsorption and immune adherence assays in conjunction with absorption tests. In contrast to the melanoma-specific antigens demonstrated previously, the surface antigens detected by these sera occurred on a broad range of nucleated cells, both normal and malignant, from human, monkey, mouse, and chicken sources. Each serum had a characteristic pattern of reactivity in absorption tests, indicating the detection of distinct antigenic systems. Two sera showed auto-, allo-, and xenoreactivity, as well as the capacity to distinguish different cell populations in the same individual. The other serum reacted with an antigen apparently universally present on nucleated cells from a variety of species, but absent on erythrocytes. As these patients had been treated with chemotherapy, this may have played a role in the emergence of these broadly reactive autoantibodies.

We thank Doctors Y. Kodera and M. A. Bean, Virginia Mason Research Center, Seattle, for kindly providing us with sera from patient BI, Doctors P. O. Livingston and T. Carey for their cooperation in obtaining specimens, and Ms. L. T. C. Li, Ms. S. H. K. Keel, Ms. A. Mendelsohn, Mr. W. V. Burton, and Mr. M. C. Taormina for their excellent technical assistance.

Received for publication 19 November 1976.

### References

1. Carey, T. E., T. Takahashi, L. A. Resnick, H. F. Oettgen, and L. J. Old. 1976. Cell surface antigens of human malignant melanoma. I. Mixed hemadsorption assays for humoral immunity to cultured autologous melanoma cells. *Proc. Natl. Acad. Sci. U. S. A.* 73:3278.
2. Shiku, H., T. Takahashi, H. F. Oettgen, and L. J. Old. 1976. Cell surface antigens of human malignant melanoma. II. Serological typing with immune adherence assays and definition of two new surface antigens. *J. Exp. Med.* 144:873.
3. Whitehouse, J. A. A., and E. J. Holborow. 1971. Smooth muscle antibody in malignant disease. *Br. Med. J.* 4:511.
4. Yoshida, T. O. 1974. Autoantibodies in the sera of various cancer patients. *Gann Monogr.* 16:63.
5. Friou, G. J. 1974. Current knowledge and concepts of the relationship of malignancy, autoimmunity and immunologic disease. *Ann. N. Y. Acad. Sci.* 230:23.
6. Gordon, P. A., P. H. Baylis, and G. W. G. Bird. 1976. Tumour-induced autoimmune haemolytic anaemia. *Br. Med. J.* 1:1569.