

## Tumor-Associated Antigens in Human Malignant Melanoma

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### INTRODUCTION

Several studies have now clearly indicated the presence of tumor-associated and tumor-specific antigens in cells of human malignant melanoma. These antigens have been identified by means of several quite different techniques, including the use of antibodies detectable against the antigens, either by means of immunofluorescence, (1-5) cytotoxicity (4, 6, 7) or by extracting the antigen and obtaining lines of precipitation in gel or cellulose acetate membrane (8). More recent studies using techniques in cellular immunology have also demonstrated antigens against which lymphocytes (7, 9-13) or macrophages (14), of patients have been shown to react. In most of the series reasonable agreement exists in that the immune response against the melanoma cells relates to the natural history of the disease but there is still considerable controversy as to the exact relationships. The likelihood that the differences can be explained on the basis of the measurement of different antigens by the different systems obviously has to be considered. It is the purpose of this paper to present evidence for a variety of antigens in human malignant melanoma and to suggest from preliminary studies that more than one immune response is, therefore, possible and that this immune response may differ with the different antigens at different stages of the disease.

### THE ANTIGENS OF MELANOMA CELLS

#### *Membrane Antigens*

Using immunofluorescence and complement-dependent cytotoxicity a surface-membrane antigen has been detected in melanoma cells which appears to be highly patient specific with little or no cross reactivity at high titer (4, 15). Also more weakly reacting membrane antigens can be detected which cross-react only at a very low intensity and virtually disappear if the serum is first diluted to one in four.

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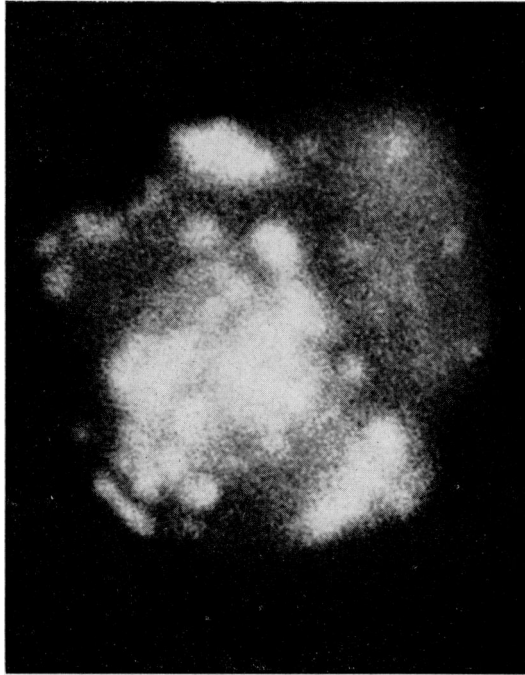


FIG. 1. Surface membrane immunofluorescence on a malignant cell viewed by epi-illumination.

We have recently developed an anti-human fetal serum by immunizing rabbits against 12-week-old human fetal material. The results have indicated that the anti-fetal serum reacts with human fetal cells but not with adult human cells. As measured by membrane immunofluorescence this serum also reacts with a number of human tumors including malignant melanoma (16). An example can be seen in Fig. 1.

#### *Cytoplasmic Antigens*

These are detected by means of immunofluorescence on cells which have been snap frozen on glass with a mixture of isopentane and liquid nitrogen (17, 18). The antigens appear to be group specific because positive sera from a number of melanoma patients cross-react widely with a number of melanoma cell preparations. The degree of intensity of the reaction may be similar in both autologous and allogeneic situations. Recent studies indicate, however, that cross-reacting allogeneic sera are of different sorts: certain sera that give positive autologous reaction, do not cross-react with other melanoma sera that have been reactive with their own cells. This is especially true in the early stage of melanoma. Again, in some recent preliminary studies which will be reported in more detail elsewhere, an antigen in the cytoplasm of ocular melanomas shows some degree of cross-reactivity but again not as wide a degree of cross-reactivity as that seen either within ocular melanomas as a group or within cutaneous melanomas as a group (19). McBride and his colleagues have recently described a nucleolar antigen in melanoma (20). In a review of our own material, we have also seen this reaction but

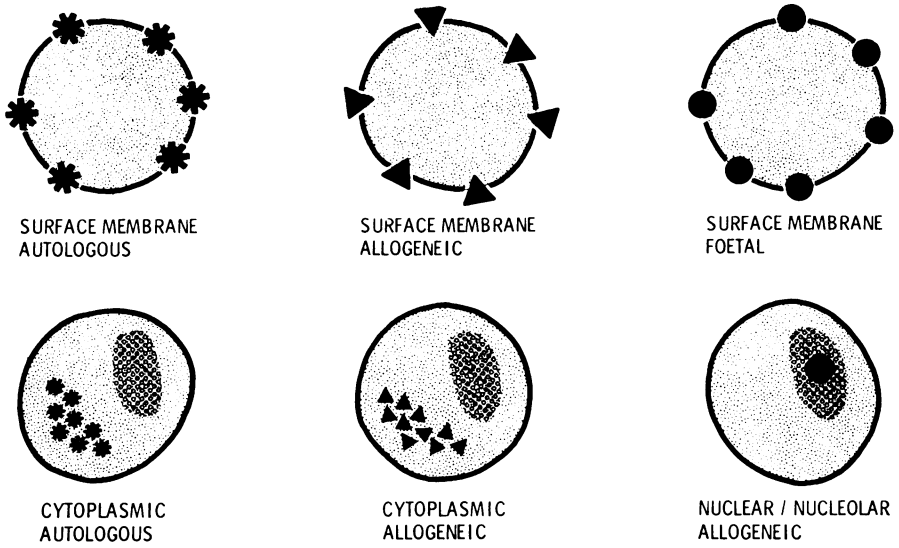


FIG. 2. Diagrammatic representation of different antigens in malignant melanoma cells.



FIG. 3. Cytoplasmic immunofluorescence in snap-frozen melanoma cell.

are not able to confirm their results. We have also noted different diffuse nuclear patterns in reactions of the sera of certain individuals to melanoma preparations which cannot be explained on the basis of antinuclear factor. This is, however, a variable reaction for which there is as yet no satisfactory explanation (See Figs. 2, 3, and 4.).

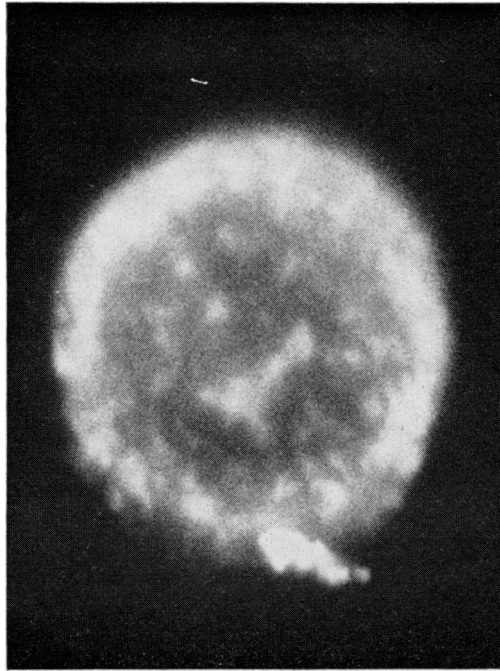


FIG. 4. Nuclear and nucleolar staining by immunofluorescence.

#### QUANTITATION OF IMMUNOFLUORESCENCE

Further light may well be thrown on these various antigens, particularly their quantitation, by the use of an epi-illumination microscope system with a photoelectric measurement device. This is a modification of that described by Ploem (21) and essentially consists of a photometer attached to an epi-illumination Nikon microscope from which a photoelectric cell and a read-out device allows one, over a narrow wave band, to measure specific fluorescence in terms of the deflection of a galvanometer. In studying the reactions of the antibodies against both cytoplasmic and membrane fluorescence in melanoma and in other tumors we have found the following: In each test 50 cells were read and the deflection of the galvanometer was recorded; the results, represented in Figs. 5, 6, and 7, show a scatter of fluorescent intensity. It can clearly be seen that the controls (phosphate-buffered saline alone or nonmelanoma serum) show a rather narrow scatter in a low-reading range (Fig. 5), but even in this a brightly fluorescent cell can occasionally be noted well into the high-reading range. Visual microscopy has identified these cells as plasma cells or lymphocytes infiltrating the original tumor preparation. This may explain the "multiple crossreactivity" which has been recorded when the tumor cells were not distinguishable from other cells carrying immunoglobulin. It might also explain how immunoglobulin is eluted from tumor cell preparation. The results of positive and negative sera against autologous and allogeneic melanoma are shown in Figs. 6 and 7 and are highly significant. Similar results can be obtained using membrane fluorescence. This method might obviate the necessity for expressing results as fluorescent indices or as titers. It might also facilitate study of the antigen-antibody interaction within an individual tumor, and separation of the cross-reacting and common antigens involved. It also gives a good indication of the

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ON CYTOPLASMIC CONTENTS OF MALIGNANT CELLS

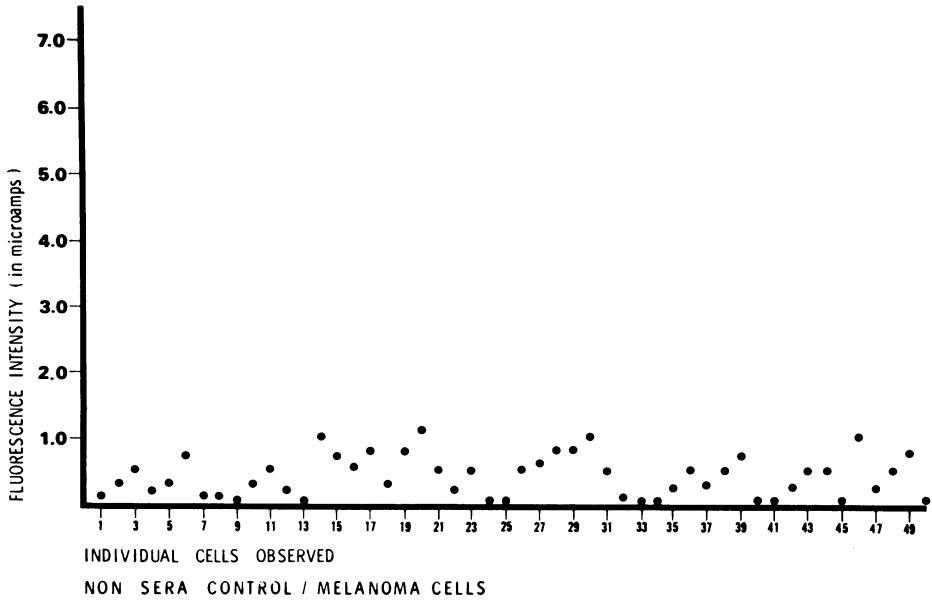


Fig. 5. Quantitation in cytoplasmic immunofluorescence: Control nonmelanoma sera.

PHOTOMETRIC QUANTITATION OF IMMUNOFLUORESCENCE  
ON CYTOPLASMIC CONTENTS OF MALIGNANT CELLS

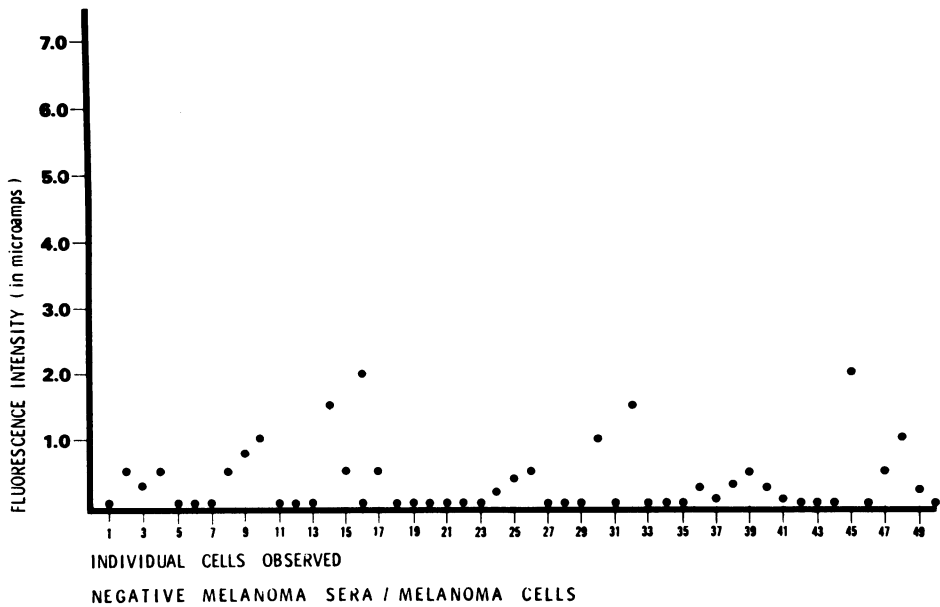


Fig. 6. Quantitation in cytoplasmic immunofluorescence: Autologous positive melanoma sera.

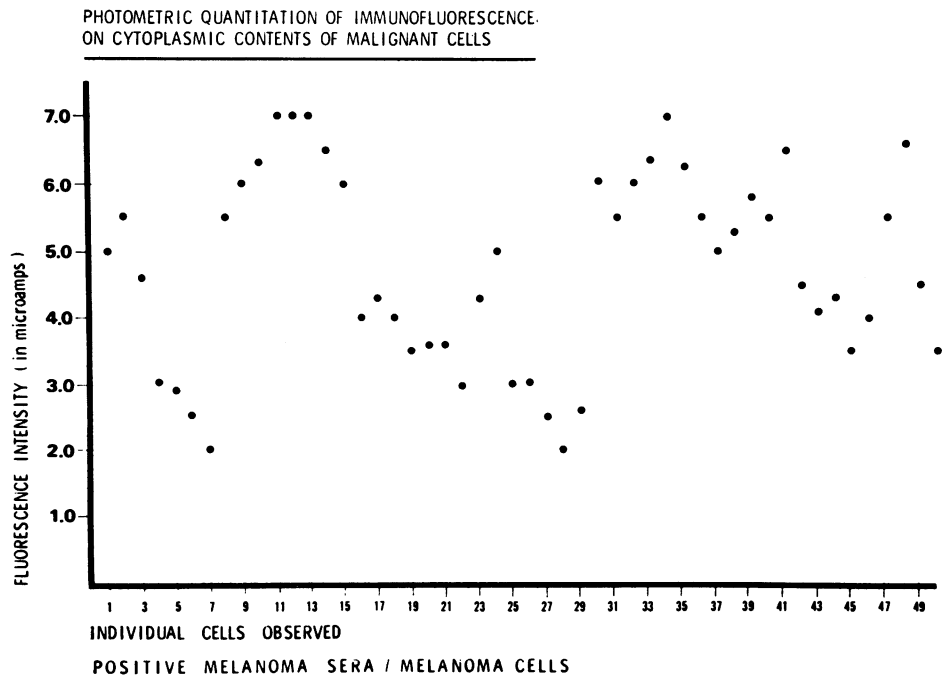


FIG. 7. Quantitation in cytoplasmic immunofluorescence: Allogeneic negative melanoma sera.

heterogeneity of the cell populations under study. These studies on freshly removed tumor cells will be compared with various cell lines which have been derived from such tumor cells to see whether particular types of tumor cell have expressed more of the various antigens than others, since this may well be of fundamental importance in the understanding of the variations of the immune response at different stages of the disease.

Finally, our present impression of the relationships between the antigens and their antibodies at various stages of the disease are summarized in Fig. 8. The autologous membrane antigen is the one we have seen most in the early stages of the tumor. Our preliminary observations in melanoma show that the autologous anticytoplasmic antibody appears next in line and may well result from local destruction of tumor and, therefore, availability of this antigen to the host in general. Finally, antibodies may be present against the allogeneic cytoplasmic antigen at a later stage of the disease, particularly if antigen has been liberated during tumor dissemination. Certainly the antibody against this allogeneic antigen may not be present even when the antibody against the autologous cytoplasmic antigen is clearly there (22). The relationship between the antigen of the cytoplasm of the ocular and cutaneous melanoma is still under investigation. The fundamental questions to be answered from such investigation are (1) what antigen appears at what stage of the disease? and (2) can an antigen alone be held responsible for the apparent fall in immune response in the disseminated phase of melanoma? We have already indicated (23) that patients can produce what appear to be either antibody-antigen complexes or even anti-idiotypic antibodies which allow the tumors to disseminate via the bloodstream. We are now investigating whether these

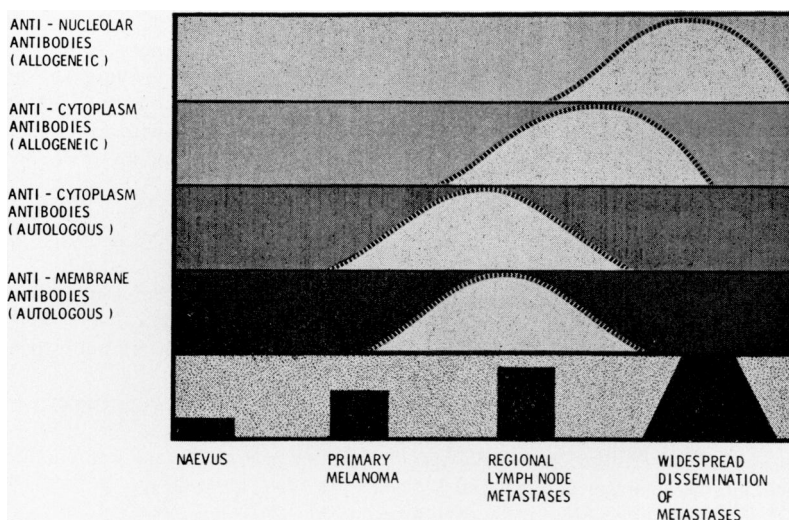


FIG. 8. Diagrammatic summary and proposed relationships between antibodies against various melanoma antigens at different states in the natural history of malignant melanoma.

various antigens and their appropriate antibodies can be measured in individual patients over a sequential period of time as the disease progresses from nevus to disseminated melanoma. So far we have been able to separate, in an individual serum, an antibody against the surface membrane component as against the cytoplasmic autologous component (18), and it is hoped by using bioabsorption column techniques that the various antigens will be obtained in a relatively pure form and then the measurement of these in the circulation will complement those studies already outlined. It will then be possible to study the relationship between humoral and cellular immune reactions against particular antigens. Therefore, before one can actually manipulate the immune response to the benefit of the patient we need to know about antigens and the reactions, both humoral and cellular, against them as well as their interactions with each other.

## REFERENCES

1. Morton, D. L., Malmgren, R. A., Holmes, E. C., and Ketcham, A. S. Demonstration of antibodies against human malignant melanoma by immunofluorescence. *Surgery* **64**, 233 (1968).
2. Muna, N. M., Marcus, S., and Smart, C. Detection by immunofluorescence of antibodies specific for human malignant melanoma cells. *Cancer* **23**, 88 (1969).
3. Rhomsdahl, M. D., and Cox, I. S. Immunofluorescent studies of antibodies against human malignant melanoma. *Surg. Forum* **10**, 126 (1969).
4. Lewis, M. G., Ikonopisov, R. L., Nairn, R. C. *et al.* Tumour specific antibodies in human malignant melanoma and their relationships to the extent of the disease. *Brit. Med. J.* **3**, 547-552 (1969).
5. Oettgen, H. F., Old, L. J., McLean, E. P., and Casswell, E. A. Delayed hypersensitivity and transplantation immunity elicited by soluble antigens of chemically induced tumours in inbred guinea pigs. *Nature (London)* **220**, 295 (1968).
6. Lewis, M. G. Malignant melanoma of the sole of the foot in Uganda. The role of pigmentation. *Brit. J. Cancer* **21**, 483 (1967).
7. Nairn, R. C., Nind, A. P. P., Guli, E. P. G., Muller, H. K., Rolland, J. M., and Minty, C. C. J. Specific immune response to human skin carcinoma. *Brit. Med. J.* **4**, 701-705 (1971).

8. Czajowski, N. P., Rosenblatt, M., Wolf, P. L., and Vasquez, J. A new method for active immunization to autologous human tumour tissue. *Lancet* **2**, 905 (1967).
9. Little, J. H. Histology and prognosis in cutaneous malignant melanoma. "Melanoma and Skin Cancer." P. 107. VCN Blight Government Printer, Sydney, Australia.
10. Currie, G. A., Lejeune, F., and Fairley, G. H. Immunization with irradiated tumour cells and specific lymphocyte cytotoxicity in malignant melanoma. *Brit. Med. J.* **2**, 305-310 (1971).
11. Jehn, U. W., Nathanson, L., Schwartz, R. S., and Skinner, M. In vitro lymphocyte stimulation by a soluble antigen from malignant melanoma. *N. Engl. J. Med.* **283**, 329-333 (1970).
12. Hellstrom, K. E., and Hellstrom, I. Some aspects of immune defense against cancer. I. In vitro studies on animal tumors. *Cancer* **28**, 1266-1268 (1971).
13. Cochrane, A. J., Jehn, U. W., and Gothoskar, B. P. Cell mediated immunity to malignant melanoma. *Lancet* **1**, 1340-1341 (1972).
14. Lejeune, F. J. Etude des Macrophages dans le Melanome Harding-Passey. *Rev. Inst. Pasteur Lyon*, **4**, 349-359 (1971).
15. Lewis, M. G., and Phillips, T. M. The specificity of surface membrane immunofluorescence in human malignant melanoma. *Int. J. Cancer* **10**, 105-111 (1972).
16. Avis, P. G. J., and Lewis, M. G. Paper in preparation.
17. Nairn, R. C. "Fluorescent Protein Tracing," 3rd edit. Livingstone, Edinburgh, 1969.
18. Phillips, T. M., and Lewis, M. G. A system of immunofluorescence in the study of tumour cells. *Rev. Eur. Etudes Clin. Biol.* **15**, 1016 (1970).
19. Federman, J. L., Lewis, M. G., Clark, W. H., Egerer, I., and Sarin, L. K. Tumour associated antibodies in the serum of ocular melanoma patients. Abstract submitted:- Amer. Acad. of Ophthalmol. Otolaryngol.
20. McBride, C. M., Bowen, J. M., and Dmochowski, L. Anti nucleolar antibodies in the sera of patients with malignant melanoma. *Surg. Forum* **23**, 92-93 (1972).
21. Ploem, J. S. The use of a vertical illuminator with interchangeable dichroic mirrors for fluorescent microscopy with incident light. *Z. Wiss. Mikrosk.* **168**, 129-142 (1967).
22. Lewis, M. G., Spitler, L., Sheikh, K. M. A., and Proctor, J. W. Paper in preparation.
23. Lewis, M. G., Phillips, T. M., Cook, K. B., and Blake, J. Possible explanation for loss of detectable antibody in patients with disseminated malignant melanoma. *Nature (London)* **232**, 52-54 (1971).
24. Lewis, M. G., and Phillips, T. M. Separation of two distinct tumour-associated antibodies in the serum of melanoma patients. *J. Nat. Cancer Inst.* **49**, 915 (1972).