Decreased Expression of HLA Class II Antigens on Human Uveal Melanoma Cells After In Vivo X-ray Irradiation

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In order to determine the presence of HLA antigens on human uveal melanomas, we tested anti-HLA monoclonal antibodies on tissue sections of these tumors. A great variety in expression of HLA class I and II antigens was present. A significantly lower expression of HLA class II antigens was present on uveal melanomas that had been irradiated before enucleation. These tumors lacked a lymphocytic infiltrate in comparison with non-irradiated tumors. These data suggest that radiotherapy affects expression of histocompatibility antigens on tumors.

SEVERAL FACTORS exert an influence on prognosis in patients with uveal melanoma, for example, tumor cell type, localization, size, and depth of scleral invasion.¹⁻³ Differences in the clinicobiological behavior between tumors as observed after enucleation may be influenced by the time interval between the development of the tumor and the time of removal, while recognition of the tumor by the immune system may play a role as well.^{4,5} HLA antigens are glycoproteins that are essential for antigen recognition by the immune system. The quantity of HLA class I and II antigens on tumor cells may influence the level of immune reactions.⁶ Uveal melanomas show heterogeneity in the level of expression of tumor-associated antigens,⁷ but it is not yet known whether such

differences are present in HLA antigen expression.

HLA class I antigens are expressed on almost all nucleated cells and function as a target to cytotoxic T cells. In the eye, these antigens have been demonstrated on the anterior border of the iris, the epithelium and external basement membrane of the ciliary body, and on the endothelial cells of the choroidal vessels.⁸ Oncogenicity of certain viruses to rodent cells⁹ was shown to depend on their capacity to decrease class I expression of infected cells, resulting in less recognition of class I negative transfected cells by T lymphocytes. Expression of class I antigens may also be essential for the elimination of virally infected cells and malignant cells in humans.

HLA class II antigens are mainly expressed on cells involved in the immune response and regulate communication between these cells and presentation of antigen to helper T cells.¹⁰ At least three different class II products have been identified, subsequently called DR, DQ, and DP, but no clear functional differences between these products have as yet been found. Class II antigens have been demonstrated on scattered cells throughout the entire uvea.⁸

Although data concerning HLA expression of uveal melanomas are lacking, data on HLA and skin melanomas are abundant. Human cutaneous melanomas show heterogeneous expression of both class I and class II antigens, with changes in expression during the course of tumor progression: HLA class I antigens are found on few common nevocellular nevi, but in most primary cutaneous melanomas.¹¹ Class II expression is not observed in nevi, but is common in cutaneous melanomas, and is associated with increased tumor thickness and early metastasis.^{12,13} Class I and II antigen expression is more pronounced in the presence of a lymphocytic infiltrate.¹¹

We know many prognostic factors in uveal

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melanomas with regard to life expectancy¹⁻³ but still are unaware of the underlying mechanisms. Immunologic factors like expression (or lack of expression) of HLA antigens may play a major role in recognition of a tumor or of early metastases.

The purpose of this study was to determine whether variable levels of expression of HLA class I and II antigens exist in human uveal melanomas. A panel of different anti-HLA monoclonal antibodies was applied to tumor specimens. HLA expression was compared to clinicopathologic characteristics of these tumors, such as tumor size, cell type, and scleral invasion, as well as to age and sex of the patient.

Material and Methods

Specimens were obtained from 27 uveal melanomas after enucleation (Table 1). Immediately after enucleation a portion of each tumor was transsclerally removed under transillumination and snap-frozen in OCT compound and stored at -70°C . The remainder of each specimen was processed for histopathologic examination.

Tumors were obtained from three locations (Universities of Amsterdam, Leiden, and Rotterdam). Nine melanomas were obtained from a location (Rotterdam) where, in the context of another investigation, eyes were irradiated on the last two days before enucleation with 4-Gy electron beam irradiation, totaling 8 Gy.

Histopathologic examination—In each case, the diagnosis was established by using conventional histologic sections stained with hematoxylin and eosin prepared from paraffin-embedded tissue, and celloidin-embedded tissue. Cell type (spindle, mixed, or epithelioid), pigmentation (absent, less than half of the cells, more than half of the cells pigmented, or heavily pigmented), mitotic frequency, lymphocytic infiltration, necrosis, tumor diameter, tumor elevation, vasoinvasive growth, and scleral invasion (absent, slight, moderate, deep, or episcleral) were determined in all of these preparations by one of us (D.W.-R.). The presence and intensity of inflammatory infiltrate (absent, scarce, or present) was assessed on 4- μm -thick toluidine blue-stained frozen sections taken immediately adjacent to the sections to be used in the immunoperoxidase procedure.

Immunoperoxidase study—Frozen sections

TABLE 1
CLINICAL AND HISTOPATHOLOGIC DATA OF 27 UVEAL MELANOMAS

PATIENT SEX, AGE (YRS)	EYE	TUMOR NO.	CELL TYPE	DIAMETER (MM)	PROMI-NENCE (MM)	SCLERAL INVASION
F, 49	RE	1*	Mixed	15.0	9.0	Absent
F, 62	LE	2*	Mixed	11.0	2.5	Absent
F, 36	RE	3†	Spindle	11.0	11.0	Deep
M, 35	LE	4†	Mixed	16.0	1.5	Deep
F, 37	LE	5†	Epithelioid	—†	—†	Absent
F, 73	LE	6†	Spindle	11.0	2.5	Slight
F, 59	LE	7†	Epithelioid	15.0	5.0	Episcleral
M, 50	LE	8†	Mixed	15.0	7.0	Episcleral
M, 53	RE	9†	Mixed	10.0	4.0	Slight
M, 64	RE	10	Mixed	16.0	9.0	Slight
F, 40	RE	11	Spindle	15.0	5.0	Deep
F, 80	LE	12	Mixed	13.0	3.0	Slight
M, 63	RE	13	Spindle	12.0	7.0	Deep
F, 57	RE	14	Mixed	11.5	5.5	Moderate
M, 63	RE	15	Spindle	14.0	9.0	Slight
F, 58	LE	16	Epithelioid	12.0	9.0	Episcleral
F, 52	LE	17	Epithelioid	22.0	9.0	Moderate
M, 67	LE	18	Spindle	15.0	8.0	—†
M, 21	RE	19	Spindle	12.0	3.5	Moderate
M, 78	RE	20	Spindle	15.0	9.0	Episcleral
F, 30	LE	21	Mixed	13.0	9.0	Moderate
M, 49	LE	22	Mixed	16.0	12.0	Deep
M, 66	RE	23	Epithelioid	17.0	12.0	Moderate
F, 62	RE	24	Mixed	16.0	6.0	Episcleral
M, 54	RE	25	Epithelioid	12.0	4.0	Deep
M, 70	LE	26	Spindle	9.0	2.0	Slight
M, 61	RE	27	Mixed	14.0	14.0	Episcleral

*Used in first study only.

†Used in both studies.

‡Could not be determined because of preparational artifacts.

4 μm thick were air-dried and fixed in acetone for ten minutes. Sections were incubated for 60 minutes at room temperature with a monoclonal antibody diluted in phosphate-buffered saline and then for 30 minutes with rabbit anti-mouse immunoglobulin-horseradish peroxidase conjugate. Between the two incubations, sections were washed three times with phosphate-buffered saline (pH 7.4). Staining was achieved by incubation of sections in an acetate buffer solution (pH 5.0) that contained 3-amino-9-ethylcarbazole, dimethylformamide, and hydrogen peroxide. Finally, the sections were washed in acetate buffer, counterstained in Mayer's hematoxylin, and coverslipped with Aquamount (Gurr, BDH Chemicals Ltd.). As a

control, a section was not incubated with a monoclonal antibody but with saline. Lack of standardization of monoclonal antibodies for both affinity and avidity may lead to differences in reactivity. In order to minimize such variability, monoclonal antibodies were always first calibrated against sections of skin melanomas with known reaction patterns.¹²

The percentage of positively staining tumor cells was assessed by light microscopy by three independent investigators as previously reported in cutaneous melanomas.¹¹ The results of the three investigators deviated maximally 10%, and average scores were used in the calculations. Positive reactions were identified by a red color, making differentiation from brown-black melanin pigment possible.

Monoclonal antibodies—Initially we tested nine tumors with three monoclonal antibodies against HLA class I, one against β_2 -microglobulin (a molecule associated with class I antigens), and six against the HLA class II antigens. Because of lack of tumor tissue, the second part of the study comprised only seven of these nine tumors, as well as 18 new ones (Table 2). One monoclonal antibody of each specificity with clear positive reactions against the tumors was used for testing against 25 melanomas.¹⁴⁻²⁰

Statistical analysis—The Spearman rank correlation test for two quantitative variables, calculating two-sided P values, or the K-samples test was applied.

TABLE 2
MONOCLONAL ANTIBODIES USED IN THIS
STUDY AND NUMBER OF TUMORS TESTED

SPECIFICITY	MONOCLONAL ANTIBODY	NO. IN FIRST STUDY	NO. IN SECOND STUDY	
			NON- IRRADIATED	IRRADIATED
HLA class I	W6/32	9	16	6
	BRL	9	16	6
	B1.23.22	9	0	0
β_2 -microglobulin	—	9	16	7
HLA class II				
DR	B8.11.2	9	0	0
DR	SV2841	9	0	0
DR- β	SV1254	9	16	9
DP	FA	8	16	9
DQ	Leu 10	9	0	0
DQ	L3.8	9	16	9

Results

In all tumors, positive staining occurred with different numbers of tumor cells, macrophages, or endothelial cells. The percentage of tumor cells staining positively was determined in areas that were clearly recognizable as consisting of malignant cells.

In the first part of the study different monoclonal antibodies against the same specificities were tested to determine whether they reacted in a similar manner. Two (BRL and B1.23.22) of the three monoclonal antibodies against HLA class I gave identical results, and showed a distinct reaction with five out of the nine tumors; the third one (W6/32) reacted with all tumors (Fig. 1). The monoclonal antibody reacting against β_2 -microglobulin showed the same or more reactivity than BRL/B1.23.22 and less reactivity than W6/32.

Monoclonal antibodies directed against HLA class II antigens reacted with less cells than the monoclonal antibodies against HLA class I antigens (Fig. 2). Staining with the three anti-HLA-DR monoclonal antibodies resulted in the same percentages: two tumors were highly DR positive, three tumors had DR antigens on one third of their cells, and the remaining four tumors had DR antigens on less than 20% of their cells. Several tumors showed different levels of expression in different areas. Anti-HLA-DP showed a similar or lower expression than DR. The two anti-HLA-DQ antibodies reacted in the same way, and expression was always lower than of DR or DP. Most tumors had less than 1% DQ positive cells.

To determine the frequency of occurrence of the antigens involved we selected one monoclonal antibody of each category and tested them on a total of 25 uveal melanomas.

Results of the monoclonal antibody tests on 25 tumors in the second part of the study are given in Figure 3. These tumors have also been used to determine the expression of cutaneous melanoma-associated antigens on uveal melanomas, which results are described elsewhere.²¹ The tumors in the second part of the study showed the same variation in HLA class I and II expression as those in the first part (Fig. 2). Levels of class I antigens and of β_2 -microglobulin were higher than the levels of HLA class II antigens. In general, DQ showed a lower expression than DR and DP.

No correlations were observed between expression of any of the antigens and any tumor

Fig. 1 (Jager and associates). Percentage of positively staining tumor cells in nine tumors. One symbol indicates one tumor. One monoclonal antibody against β_2 -microglobulin and three against HLA class I were used.

Fig. 2 (Jager and associates). Percentages of tumor cells staining positively for HLA class II antigens -DR (three monoclonals), -DP (one monoclonal), and -DQ (two monoclonals).

Fig. 3 (Jager and associates). Percentages of positive staining with different monoclonal antibodies in tumor cells of 25 uveal melanomas. One dot represents one tumor. Because of lack of tissue some tumors were not tested with all monoclonal antibodies.

factors, such as diameter, elevation, scleral invasion, tumor cell type, and age and sex of the patient. No correlation was observed between external electron beam irradiation with 8 Gy before enucleation and β_2 -microglobulin or class I expression (Fig. 4). A strong correlation, however, was observed between irradiation of the eye on the two days before enucleation, absence of class II antigens, and absence of infiltrate in the tumor. All irradiated tumors but one showed a significantly lower expression of class II HLA antigen in comparison with the nonirradiated ones (Fig. 5, Table 3). The respective P values were as follows: DR, $P = .003$; DP, $P = .021$, and DQ, $P = .006$. There also was a significant difference in lymphocytic infiltrate between irradiated and nonirradiated tumors ($P < .001$).

Discussion

The uveal melanomas tested showed a great variability in expression of HLA class I and II antigens. The reason for this phenomenon is unknown: expression may have been caused by intrinsic factors such as the state of differentia-

tion of the tumor, oncogenes, and cell type, or expression may have been caused by external factors such as the presence of lymphokines, for example, interferon. In cutaneous melanomas a relationship was observed between expression of the HLA antigens and the presence of a lymphocytic infiltrate.¹¹ In our study HLA class I antigens were expressed on most tumors and the average percentage of expression was higher than of class II. The discrepancy between W6/32 and the other two anti-HLA class I monoclonal antibodies and anti- β_2 -microglobulin was obvious but not understood. There was a decreasing expression of DR, DP, and DQ antigens, respectively. Because variations in avidity and affinity between monoclonal antibodies may also cause differences in staining, one has to be careful with the interpretation of such data. However, our monoclonal antibodies with the same specificities showed similar reaction patterns, and the same hierarchy in expression of DR, DP, and DQ has also been reported for leukemic cells²² and normal class II positive tissues.²³ In our study this sequence was not only observed in nonirradiated but also in the irradiated samples.

In nonirradiated tumors a great variety in

Fig. 4 (Jager and associates). Tumors without (open circles) and with (closed circles) preirradiation, showing similar levels of HLA class I antigen expression. Dotted lines indicate averages.

Fig. 5 (Jager and associates). Tumors without (open circles) and with (closed circles) preirradiation, showing different levels of HLA class II antigen expression. Because of lack of tissue some tumors were not tested with all monoclonal antibodies. Dotted lines indicate averages.

TABLE 3
CORRELATIONS BETWEEN HLA CLASS II EXPRESSION
AND CLINICOPATHOLOGIC FACTORS

	HLA-DP	HLA-DQ	INFILTRATE	RADIOTHERAPY
HLA-DR	0.000	0.001	0.082	0.003
HLA-DP	×	0.000	0.007	0.021
HLA-DQ		×	0.001	0.006
Infiltrate			×	0.000

expression of HLA class I and II antigens could be observed. Only expression of DQ and not of class I, DR, or DP was associated with the presence of a lymphocytic infiltrate. This was primarily caused by several tumors that did have a local infiltrate but did not express the antigens involved on most of the tumor cells. In such tumors, a local class II positive reaction (on 3% to 5% of the tumor cells) could be observed around vessels and around lymphocytic cells. These findings suggest that some relationship exists between the presence of an infiltrate and expression of at least one of the class II antigens in uveal melanomas.

An interesting coincidental finding was the lower level of expression of all three class II antigens in the eyes that had received irradiation before enucleation (Fig. 4). Samples from irradiated tumors were obtained from one center, and no differences in methods of enucleation or treatment of the specimens were noticed between centers. Since irradiated and nonirradiated tumors showed the same expression of β_2 -microglobulin, class I, and various melanoma-associated antigens,²⁰ we think that sampling errors or selection bias probably do not play a role here. Although only a relatively small number of tumors was tested, our data indicate that preenucleation radiotherapy may reduce the expression of HLA class II antigens.

The observed influence of irradiation may be caused by a direct influence on tumor cells or indirectly by an effect on lymphocytes. A distinct difference in the extent of lymphocytic infiltrate was observed between irradiated and nonirradiated tumors. Whereas in the total material (25 tumors) the association between presence of infiltrate and of DP and DQ expression was significant, the association between infiltrate and DR expression was not. This may be because several nonirradiated tumors that lacked infiltrate were markedly positive for DR, while they lacked DP and DQ. The expression of HLA-DR could therefore also be related to the process of malignant transformation per se,

and irradiation might abrogate the production of HLA glycoproteins. Preliminary data on HLA expression after irradiation of Epstein-Barr virus transformed B cell lines do not support a direct influence of irradiation (personal communication, F. H. J. Claas, M.D., Department of Immunohaematology and Bloodbank, University Hospital, Leiden, The Netherlands). Conversely, lymphocytes produce interferon, which stimulates expression of HLA class II antigens on all kinds of cells, for example, on melanoma cell lines.²⁴ Transplantation experiments with melanoma cells into nude mice also support this view: class II positive melanoma cell lines lost their class II expression after implantation in these mice, which lack the specific lymphokines that could stimulate HLA expression.²⁵ Reduction of lymphocytic infiltration by irradiation may lead to a decrease in stimulation of HLA class II expression. Since an association exists between irradiation, absence of lymphocytic infiltrate, and lack of HLA-DQ and -DP antigens, we favor the hypothesis that in choroidal melanoma, lymphocytes stimulate expression of HLA class II antigens on tumor cells.

It may be that the level of HLA-antigen expression in uveal melanomas will become immunologically relevant only after extraocular growth of the tumor: it was observed that patients whose melanoma had penetrated the sclera had a higher cellular and serologic reactivity against the tumor cells in vitro.²⁶ This could suggest that scleral penetration makes recognition by the immune system possible, since recognition depends on the presence of HLA class I and II antigens. However, in our study we did not find any correlation between HLA expression and scleral invasion. Since recognition by T cells of all antigens, including tumor-specific antigens, is probably HLA-dependent, not only the immune response to HLA antigens but to all antigens may vary with the level of HLA antigens on the tumor. It may be worthwhile to compare cellular and serologic reactivity against uveal melanomas with the level of expression of HLA and tumor-associated antigens on melanoma cells. It may also be interesting to study the absence or presence of an infiltrate in tumors after ruthenium application. However, the influence of ionizing irradiation on the immune system is complex.²⁷ In general, irradiation causes a decrease in resistance, for example, against pathogens and transplanted grafts. For instance, total body irradiation is used to prevent graft vs host reactions after bone marrow transplanta-

tion. One observation that irradiation may affect class II expression was described,⁶ that is, a decrease in expression of class II on mouse kidneys was found after total body irradiation of the mice. T-cell activation occurs after contact between T cells and macrophages or other antigen presenting cells, carrying both class II and antigen on their surface.¹⁰ Irradiation may decrease expression of HLA class II antigens and therefore lower immunologic recognition in a manner similar to corticosteroids and cyclosporine, both of which decrease expression of HLA class II antigens.²⁷ It may be worthwhile to study the expression of HLA class II antigens on lymphocytes and in normal tissues of irradiated patients. We suggest that not only the direct influence of irradiation on lymphocytes but also the effect of lymphocytes on HLA expression may contribute to the increased sensitivity of irradiated individuals to infections. The abrogation of HLA class II antigen expression by ionizing irradiation offers a new explanation for the immunosuppressive effect of irradiation. Irradiation of melanomas may have a destructive effect on dividing cells or influence immunologic recognition. Which factor has more influence on the tumor will require careful follow-up to determine a possible influence of irradiation on uveal melanomas and prognosis.

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References

1. Gamel, J. W., McLean, I. W., Foster, W. D., and Zimmerman, L. E.: Uveal melanomas. Correlation of cytologic features with prognosis. *Cancer* 41:1897, 1978.
2. Jensen, O. A.: Malignant melanomas of the human uvea. *Acta Ophthalmol.* 60:161, 1982.
3. Pach, J. M., Robertson, D. M., Taney, B. S., Martin, J. A., Campbell, R. J., and O'Brien, P. C.: Prognostic factors in choroidal and ciliary body melanoma with extrascleral extension. *Am. J. Ophthalmol.* 101:325, 1986.
4. Priluck, I. A., Robertson, D. M., Pritchard, D. J., and Ilstrup, D. M.: Immune responsiveness in patients with choroidal malignant melanoma. *Am. J. Ophthalmol.* 87:215, 1979.
5. Kaplan, H. J.: Lymphocyte subpopulations in uveal malignant melanoma. *Am. J. Ophthalmol.* 101:483, 1986.
6. Halloran, P. F., Wadgymar, A., and Autenried, P.: The regulation of expression of major histocompatibility complex products. *Transplantation* 41:413, 1986.
7. Folberg, R., Donoso, L. A., Atkinson, B. F., Ernst, C. S., Herlyn, M., and Arbizo, V. V.: An antimelanoma monoclonal antibody and the histopathology of uveal melanomas. *Arch. Ophthalmol.* 103:275, 1985.
8. Bakker, M., and Kijlstra, A.: The expression of HLA-antigens in the human anterior uvea. *Curr. Eye Res.* 45:599, 1985.
9. Schrier, P. I., Bernards, R., Vaessen, R. T. M. J., Houweling, A., and van der Eb, A. J.: Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. *Nature* 305:771, 1983.
10. Doherty, P. C., Knowles, B. B., and Wettstein, P. J.: Immunological surveillance of tumors in the context of a major histocompatibility complex restriction of T cell function. *Adv. Cancer Res.* 42:1, 1984.
11. Ruiter, D. J., Bhan, A. N., Harrist, T. J., Sober, A. J., and Mihm, M. C., Jr.: Major histocompatibility antigens and mononuclear inflammatory infiltrate in benign nevocellular proliferations and malignant melanoma. *J. Immunol.* 129:2808, 1982.
12. Ruiter, D. J., Bergman, W., Welvaart, K., Scheffer, E., van Vloten, W. A., Russo, C., and Ferrone, S.: Immunohistochemical analysis of malignant melanomas and nevocellular nevi with monoclonal antibodies to distinct monomorphic determinants of HLA antigens. *Cancer Res.* 44:3930, 1984.
13. Bröcker, E.-B., Suter, L., Brüggen, J., Ruiter, D. J., Macher, E., and Sorg, C.: Phenotypic dynamics of tumor progression in human malignant melanoma. *Int. J. Cancer* 36:29, 1985.
14. Parham, P., Barnstable, C. J., and Bodmer, W. F.: Use of a monoclonal antibody (W6/32) in structural studies of HLA-A,B,C antigens. *J. Immunol.* 123:342, 1979.
15. Rebai, N., and Malissen, B.: Structural and genetic analyses of HLA class I molecules using monoclonal xeno antibodies. *Tissue Antigens* 22:107, 1983.
16. Quaranta, V., Indiveri, F., Glassy, M. C., Ng, A., Russo, C., Molinaro, G. A., Pellegrino, M. A., and Ferrone, S.: Serological, functional and immunohistochemical characterization of a monoclonal antibody

(Moab Q2/70) to human Ia-like antigens. *Hum. Immunol.* 3:211, 1980.

17. Quaranta, V., Walker, L. E., Pellegrino, M. A., and Ferrone, S.: Purification of immunologically functional subsets of human Ia-like antigens on a monoclonal antibody (Q5/13) immuno-adsorbent. *J. Immunol.* 125:1421, 1980.

18. Watson, A., DeMars, R., Trowbridge, I. S., and Bach, F. H.: Detection of a novel human class II HLA antigen. *Nature* 304:358, 1983.

19. Chen, Y.-X., Evans, R. L., Pollack, M. S., Lanier, L. L., Phillips, J. H., Rousso, C., Warner, N. L., and Brodsky, F. M.: Characterization and expression of the HLA-DC antigens defined by anti-Leu 10. *Hum. Immunol.* 10:221, 1984.

20. Spits, H., Borst, J., Giphart, M., Coligan, M., Terhorst, C., and de Vries, J. E.: HLA-DC antigens can serve as recognition elements for human cytotoxic T lymphocytes. *Eur. J. Immunol.* 14:299, 1984.

21. van der Pol, J. P., Jager, M. J., de Wolff-Rouendaal, D., Ringens, P. J., Vennegoor, C., and Ruiters, D. J.: Heterogeneous expression of melanoma-associated antigens in uveal melanomas. *Curr. Eye Res.* 6:757, 1987.

22. Guy, W., Ritchie, A. W. S., van Heyningen, V., and Dewar, A. E.: Further serological characterization of the heterogeneity of expression of human MHC class II antigens. *Chronic lymphocytic leu-*

caemia cells and monocytes differentially express DR and DC antigens. Disease Markers 1:249, 1983.

23. Müller, C., Ziegler, A., Müller, C., Hadam, M., Waller, H.-D., Wernet, P., and Müller, G.: Divergent expression of HLA-DC7, MB1-DR, and SB region products on normal and pathological tissues as detected by monoclonal antibodies. *Immunobiology* 169:228, 1985.

24. Kameyama, K., Takezaki, S., Kanzaki, T., and Nishiyama, S.: HLA-DR and melanoma-associated antigen (p97) expression during the cell cycle in human melanoma cell lines, and the effects of recombinant gamma-interferon. Two-color flow cytometric analysis. *J. Invest. Dermatol.* 87:318, 1986.

25. van den Brink, M. R. M., van Muyen, G. N. P., Guyt, C. J., Schrier, P. I., and Ruiters, D. J.: Suppression of HLA-class II expression in human melanoma cells xenografted to nude mice. *J. Pathol.* 149:237A, 1986.

26. Cochran, A. J., Foulds, W. S., Damato, R. E., Trope, G. E., Morrison, L., and Lee, W. R.: Assessment of immunological techniques in the diagnosis and prognosis of ocular malignant melanoma. *Br. J. Ophthalmol.* 69:171, 1985.

27. Anderson, R. E., and Warner, M. L.: Ionizing radiation and the immune response. *Adv. Immunol.* 24:215, 1976.

OPHTHALMIC MINIATURE

She had the misfortune, on one of these pilgrimages during which she did not like to be disturbed, to meet on the beach a lady from Combray, accompanied by her daughters. Her name was, I think, Mme. Poussin. But among ourselves we always referred to her as "Just You Wait," for it was by the perpetual repetition of this phrase that she warned her daughters of the evils that they were laying up for themselves, saying for instance if one of them was rubbing her eyes: "Just you wait until you go and get ophthalmia."

Marcel Proust, *Remembrance of Things Past*
Translated by C. K. Scott Moncrieff and Terence Kilmartin
New York, Random House, 1981