Thymus-derived lymphocyte enumeration in patients with uveal malignant melanoma

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SUMMARY Thymus-derived lymphocytes (T lymphocytes) were enumerated in patients with uveal malignant melanoma. Two T-lymphocyte subpopulations were determined, the active rosette forming cells (A-RFC) and the total rosette forming cells (T-RFC). Subjects were divided into the following groups: (a) pretreatment patients, (b) patients treated by enucleation, (c) patients treated by photocoagulation, (d) patients treated by cobalt plaque radiotherapy, (e) patients treated by enucleation who developed clinically detectable metastasis, and (f) normal controls. There were no differences in the numbers of A-RFC or T-RFC in the control population, pretreatment patients, and those treated in the different ways. Statistically significant depressions of A-RFC and T-RFC levels were seen in patients with metastatic lesions, suggesting that they had an impairment of immunocompetence, as measured by T-lymphocyte rosette formation.

Immune surveillance of solid tumours is generally accepted to be a function of the cell-mediated immune system. The ability of malignant cells to escape surveillance has been postulated to be due to a number of mechanisms such as the 'shedding' of tumour antigens or of other tumour products which can cause the suppression of the host's immune system.¹ Cell-mediated immune responses have been demonstrated by in-vitro and in-vivo methods in patients with uveal malignant melanoma.^{2,3}

In this study active rosette forming cells (A-RFC) and total rosette forming cells (T-RFC) were enumerated as a measure of cell-mediated immunity because they are reported to correlate well with cell-mediated immunity.⁴ A-RFC represent a special subset of T lymphocytes that have a higher affinity for sheep erythrocytes (E) and have a more consistent relationship to active immunity than do T-RFC.⁵ A-RFC probably represent 'activated' T lymphocytes, which are involved in ongoing immune responses.⁴

Patients and methods

Patients. Eighty patients with the clinical diagnosis of uveal malignant melanoma who had not been treated were examined at the Oncology Service of the Wills Eye Hospital by one of the authors (J.A.S.) between 1977 and 1979. Therapy was provided according to established procedures.⁶ T lymphocytes were enumerated on these 80 patients. A post-therapy group consisted of the 80 pretreatment patients and an additional 24 patients who had their A-RFC and T-RFC evaluated after therapy. The patients in the post-therapy group included 62 patients who had been treated by enucleation, 15 patients treated by photocoagulation, and 27 patients treated by cobalt-60 plaque radiotherapy.⁷ During follow-up nine of the patients treated by enucleation developed metastasis confirmed either by biopsy, liver and bone scans, or necropsy. Patients receiving immunotherapy or chemotherapy were not included in this study. Normal controls consisted of 100 employees and volunteers without apparent disease or immunological depression.

Lymphocyte preparation. Ethylenediamine tetraacetic acid (EDTA) anticoagulated blood was

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separated on a Ficoll-hypaque discontinuous gradient. The lymphocyte layer was washed to remove thrombocytes. Viability by trypan blue exclusion was usually greater than 95%. Purity determined after Wright's staining indicated that more than 95% of the cells were mononuclear leucocytes.

Rosette formation. Lymphocytes (0.1 ml of 5×10^6 cells/ml of Hanks's balanced salt solution, HBSS) was mixed in a $12 \times 75 \text{ mm}$ snap-cap plastic tube with an equal volume of sheep E (0.05%) and $20 \mu\text{l}$ of absorbed normal human serum. After 5 minutes at 37°C the tube was centrifuged at 100 g for 5 minutes. A-RFC were determined immediately and T-RFC were determined after an additional 90-minute incubation at 4°C according to Wybran *et al.*⁸

Statistical comparisons. Results were compared by Student's *t* test. Probabilities less than 0.05 were considered significant.

Results

The numbers of A-RFC and T-RFC enumerated for the pretherapy patients and the controls are presented in Table 1. Overall no statistical difference was found between the two groups. During the course of the study no significant changes in the number of rosette forming cells were found in the patients treated by enucleation, photocoagulation, or cobalt plaque irradiation (Table 2). On the other hand those nine patients who developed metastatic melanoma had significantly ($p < 0.001$) decreased levels of A-RFC and T-RFC. Serial enumerations of the two rosette forming cell populations in Fig. 1 showed decreasing levels of A-RFC and T-RFC even before the clinical detection of metastatic disease.

Discussion

The loss of immunocompetence as measured by rosette forming cells in the general circulation has been previously described in other malignancies at the time of diagnosis.^{9,10} In this study we found no statistically significant differences in A-RFC and T-RFC levels at the time of diagnosis in 80 patients with malignant melanoma of the uvea (Table 1).

Table 1 Rosette forming cells in pretherapy patients with uveal malignant melanoma and in control patients

Diagnosis	Number	% Rosette forming cells	
		Active	Total
Melanoma	80	24.39 ± 11.48	55.84 ± 12.53
Controls	100	24.78 ± 10.66	55.89 ± 10.75

± = Standard deviation.

Table 2 Rosette forming cells in patients with uveal malignant melanoma after treatment

Treatment	Number	% Rosette forming cells	
		Active	Total
Enucleation	62	22.82 ± 10.13	52.45 ± 12.35
Photocoagulation	15	20.53 ± 11.54	53.80 ± 7.86
Cobalt plaque	27	22.56 ± 11.84	53.93 ± 12.00

Values indicate the average and one standard deviation of the most recent follow-up data for each patient.

Recently several authors have suggested that an increased mortality rate following enucleation may be due to several possible mechanisms, including tumour dispersion during surgery, enucleation selectively given to those patients with large or rapidly growing tumours, or immunosuppression following surgery and anaesthetic.¹¹⁻¹⁴ To assess the immunocompetence of patients after therapy for their uveal malignant melanoma we compared the numbers of A-RFC and T-RFC in patients treated in different ways. We could find no differences in the numbers of A-RFC or T-RFC in the patients treated by enucleation, photocoagulation or cobalt plaque irradiation (Table 2). Our results suggest that, if indeed these forms of therapy can induce an immunosuppression, it does not appear to be detectable with assays such as A-RFC or T-RFC enumeration.

Metastasis in other cancers has been associated with immunosuppression related to depressed numbers of rosette forming cells.¹⁵ Our study extends these observations to uveal malignant melanoma. We have detected depressed numbers of A-RFC and T-RFC as much as three months prior to the clinical detection of or death due to metastasis (Table 3, Fig. 1). In addition decreased T cells have been reported in patients with systemic metastases from uveal melanoma by a different rosetting technique.¹⁶

From the results of our study we do not believe that there is any diagnostic value in enumerating A-RFC and T-RFC lymphocytes in the initial evaluation of patients with uveal malignant melanoma. A-RFC and

Table 3 Rosette forming cells in patients with metastasis from uveal malignant melanoma

Diagnosis	Number	% Rosette forming cells	
		Active	Total
Melanoma	9*	$14.22 \pm 5.79^\dagger$	$39.89 \pm 11.23^\dagger$
Control	100	24.78 ± 10.66	55.89 ± 10.75

*Rosette forming cells were assayed 3.11 ± 3.49 months prior to the clinical detection of metastasis or death.

† $p < 0.001$ compared with controls or pretherapy patients.

Fig. 1 Serial T lymphocyte levels of four patients with uveal malignant melanoma treated by enucleation at time zero. Total rosette forming cells (T-RFC) are represented by solid symbols. The average and 1 standard deviation from the mean for 100 normal volunteers is seen to the right (T). Active rosette forming cells (A-RFC) are represented by open symbols. The average and 1 standard deviation from the mean for 100 normal volunteers is seen to the right (A). T-RFC and A-RFC levels below their respective dashed lines are considered depressed. Symbols at the top of the Figure indicate when the patient died of metastatic disease.

T-RFC enumeration may have some prognostic value, since depression can occur many months before the clinical detection of metastatic disease. Functional lymphocyte tests, such as lymphoblast transformation,^{17,18} may be more sensitive than A-RFC and T-RFC enumeration as a measure of immunocompetence, while other laboratory adjuncts that do not depend on host immunocompetence, such as serum gamma-glutamyl transpeptidase,¹⁹ may be more sensitive than rosette formation in detecting metastasis.

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