Halo nevus

Competent surveillance of potential melanoma?

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The halo nevus is characterized histologically by a dense lymphocytic infiltrate enveloping the cells of a compound nevus. Clinically the nevus develops a depigmented outer ring in the skin surrounding it (*Figure*). The nevus itself often disappears spontaneously and the depigmented halo usually repigments. Numerous mechanisms have been postulated for this phenomenon of spontaneous regression of a benign tumor.[1] Results of recent studies by Copeman et al[2] implicated circulating antibody as the mechanism responsible for regression of the halo nevus. The conclusions are based on the demonstration of antibodies in the sera of some halo nevi patients to the cytoplasm of malignant melanoma cells.

Immune response occurs by two mechanisms, cellular (T-cell) and humoral (B-cell). Cell-mediated immunity is primarily responsible for tumor and transplant rejection, not humoral immunity (antibody).

Methods

Studies of tumor immunity were performed by the cell inhibition (CI) (microcytotoxicity) assay described by the Hellströms[3] with slight modifications. Target cells were derived from melanoma cell lines grown in vitro in tissue culture starting with tumor tissue. At the time of testing, these cells had gone

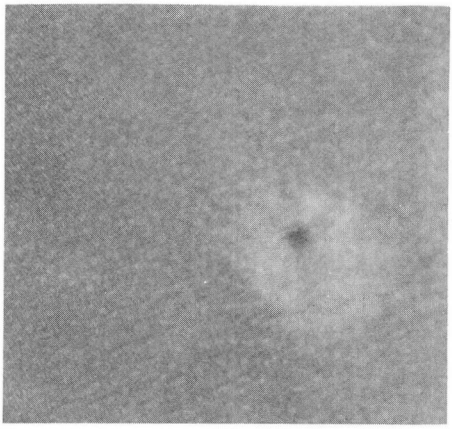

Figure. Photograph of halo nevus.

through 5 to 15 passages. Since it was not possible to grow each patient's tumor cells in every test, the target cells were allogeneic with respect to the patient's lymphocytes. Approximately 100 cells were seeded into each well of a Falcon No. 3040 microtest plate, and on the following day the cells were exposed to combinations of blood lymphocytes and sera from patients and controls. The tumor cells were known not to divide during this initial incubation period. All combinations were tested in six to eight replicate wells. Lymphocytes were obtained from heparinized peripheral blood by the Ficoll-Hypaque method of Böyum.[4] Sera were inactivated at 56C for 30 minutes and diluted 1:5 for testing. Target cells were incubated with diluted sera prior to addition of 1×10^5 lymphocytes per well. After a 40-hour incubation period, plates were stained with crystal violet, and surviving target cells were counted by microscopic examination. Controls included lymphocytes from a normal individual (previously tested and known to be unreactive) reacting with the same melanoma target cells, and patient's lymphocytes reacting with normal human fibroblasts and also with a different malignant tumor cell line (e.g., renal cell carcinoma).

Reduction of surviving target cells in the presence of lymphocytes compared to the number of target cells in control wells was expressed as percent CI (due to lymphocytes or serum cytotoxicity). Serum blocking factor was determined by comparing the percent CI of known reactive lymphocytes (from previously tested patient) in the presence of the patient's serum. The difference was expressed as percent blocking, and indicated that the presence of test serum abrogated lymphocyte killing of target cells.

All data were placed into a computer and the average, mean standard deviation, and standard error were determined; the results were expressed as percent CI or percent blocking; p values <0.01 in comparison with the controls were considered significant.

Demonstration of anticytoplasmic antibody to melanoma cell cytoplasm was by indirect immunofluorescent technique described by Copeman et al.[2] Snap-frozen smears from melanoma tissue cultures were reacted with patients' sera and then stained with fluorescein-labelled antihuman gamma globulin obtained from Hyland Laboratory (La Mesa, California). All sera were diluted 1:4 and appropriate controls were run simultaneously.

Results

During 1973 and 1974, 45 assays in melanoma patients and 47 assays in normal controls were performed. The mean CI for normal controls was 26.4%. The mean CI for all melanoma patients was 51.2%.

If one compares the number of patients with melanoma to control patients tested in 1973 and 1974 at cytotoxicity levels greater than 40% CI, there were 32 of 45 (71.1%) cases of melanoma and 16 of 47 (34.0%) normals; this is a statisti-

cally significant difference ($p < .001$). For the same comparison at levels greater than 50% CI, there were 22 of 45 (48.9%) melanoma and 11 of 47 (23.4%) normals, which is also statistically significant ($p < .001$).

The 11 patients tested could be classified into two groups (*Table*): (1) Patients with high percent CI and no blocking; (2) patients with low percent CI and serum blocking. The clinical course correlated with the two groupings also. Those patients with higher percent CI had central nevi which were obviously regressing clinically in size and pigmentation. Four of the six had almost entirely repigmented the halo. Patients in group 2 had halo nevi in which the central nevus and halo persisted unchanged (1 to 4 years). Four of five had a serum factor which blocked the ability of lymphocytes to kill melanoma cells in culture.

The presence or absence of circulating antibody to melanoma cell cytoplasm did not correlate with the clinical course, percent CI, or blocking activity of the serum.

Case 1 was of particular interest and significance. The patient serendipitously served as a normal control for the tissue culture laboratory in 1971, and at that time showed no percent CI to melanoma cells. In 1973 it was noted that a halo nevus had developed, and when the patient was retested at that time the CI was 93%.

Discussion

The halo nevus often undergoes obvious clinical regression in which the central nevus disappears and the associated halo repigments, eventually leaving the patient and clinician uncertain as to where the lesion existed. In some cases, however, the central nevus and the depigmented halo persist clinically unchanged.

It could be argued that the lesions which were clinically persistent might be histologically "burnt out" and still have a central elevated papule and depigmented halo. Whatever the cause of the observed clinical inactivity, either lack of regression of the central nevus or a "burnt out" one, we found there was an associated low percent CI. The phenomenon of active clinical regression was associated with higher percent CI. Biopsies were not done on patients in our series because we believed that would eliminate them from further studies of the natural course of the lesion.

The cell-mediated immune response

Table. Clinico-immunologic correlations in patients with halo nevus

Case	Age	Sex	Duration of lesion (yr)	Status of nevus	CI %	Blocking %	Melanoma cell cytoplasmic immunofluorescence
1	31	W/M	1	Regressing	93	0	+
2	21	W/F	1	Regressing?	90	85	0
3	31	W/M	4	Regressing	65	0	+
4	15	W/M	1	Regressing	38	0	+
5	18	W/M	1	Regressing	36	0	0
6	28	W/M	2	Regressing	33	0	0
7	16	W/F	2	Persisting	21	0	+
8	10	W/F	2	Persisting	21	26	0
9	12	W/F	1	Persisting	12	88	0
10	17	W/M	4	Persisting	11	100	0
11	11	W/F	1	Persisting	5	89	+

may well be policing potential malignancies in each of us almost daily. Immune competence decreases significantly with age, especially after age 50.[5] The average age of patients in whom halo nevi develop is about 18 years,[1] whereas those in whom melanoma develop are older than 50 years.[6] Most halo nevi develop in the first 3 decades of life when incidence of malignancy is lowest. It is possible that halo nevi develop in young, immunologically competent individuals when a potentially malignant change occurs. Early malignant change may not be detected by the lymphocytes of older persons, and their response may be too little or too late to prevent a clinical melanoma.

Whimster[7] pointed out that there are other explanations for the phenomenon. Either lymphocyte cytotoxicity or circulating antibody to melanoma cells logically could be on the basis of exposure of embryologically common antigens to the immune system through death and destruction of melanocytes or nevus cells from causes other than malignancy (e.g., ultraviolet light, radiation, chemicals, "spontaneous").

Jacobs et al,[5] by electron microscopy, studied nine halo nevi in various stages of regression. Their findings of lymphocytes, monocytes, and plasma cell infiltration of the tumor followed by vacuolar cytolysis of the nevus cells support the concept of a cell-mediated immune reaction in regressing halo nevi.

Our studies of 11 cases of halo nevi show that cellular cytotoxicity against melanoma cells may be more important than humoral antibodies. Regardless of what causes the lymphocytes of patients with halo nevi to kill melanoma cells in vitro, the fact is that they do. Possibly, if work with lymphocyte "transfer factor" or other such concepts of immunotherapy prove successful, the lymphocytes from patients with actively regressing halo nevi might serve as a reservoir for "factors" which could restore immune competence to patients with melanoma who have lost that ability.

Summary

The halo nevus is a compound nevus which in many cases spontaneously regresses. Tumor CI studies were done with the lymphocytes of 11 patients who had halo nevi to determine if there was cytotoxicity to melanoma cell cultures.

Six patients in whom the central nevus was actually regressing had significant CI (33% to 98%). Four patients in whom the central nevus persisted unchanged had a serum "blocking factor" (26% to 100%) with concomitant low CI (5% to 20%). The remaining patient had 21% CI, no blocking, and clinical nonregression of the nevus.

Circulating antibody to the cytoplasm of melanoma cells was studied by indirect immunofluorescent technique. The results were inconsistently positive and did not correlate with clinical regression or nonregression of the central nevus.

We conclude that (1) regression of the central nevus is a cell-mediated event; (2) antibody to melanoma cell cytoplasm is secondary, most likely occurring after nevus cell destruction by lymphocytes; (3) persistence of the central mole in some cases may be due to serum blocking factors.

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