

Cell-mediated Immunity of Patients who Have Had Basal Cell Carcinomas*

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The cell-mediated immunity of patients who had basal cell carcinomas (BCCs) removed was studied by measuring cutaneous delayed hypersensitivity reactions to recall antigens (Multitest CMI, Pasteur-Merieux), and by measuring lymphocyte counts and subsets. One group of patients had multiple BCCs (3 or more) removed and were considered to have a high risk of new BCC formation. The other group consisted of patients who had one BCC and had not developed another within 5 years; these were considered to have a low risk of new BCC formation. The low-risk patients had significantly larger cutaneous reactions to recall antigens ($p < 0.05$) and significantly fewer were anergic ($p < 0.01$). There was a correlation between smaller cutaneous reactions and increasing numbers of BCCs ($p < 0.05$). There was no significant difference between the groups in lymphocyte counts or subsets, but the low-risk patients had a significantly higher CD4:CD8 ratio ($p < 0.05$) than the high-risk group. The Multitest CMI test can be used to determine which patients are at risk of developing many BCCs.

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People who have had a skin cancer run a considerable risk of developing another. Prospective studies in North America found that between 36 and 50% of patients develop another skin cancer within 5 years (1,2). The risk is higher in Australia, where 60% of patients developed another skin cancer within 3 years. However, the risk varied among the patients. Those who have had multiple skin cancers (3 or more) were significantly more likely to develop new tumours than those who have had only one (3). This was similar to the finding of Epstein in California who followed patients for 1 year. He found that the greater number of skin cancers a patient had, the greater was the risk of a new skin cancer developing (4). Studies of HLA frequencies in skin cancer patients have suggested that immuno-genetic factors are involved in the genesis of multiple skin cancers. HLA DR1 has been associated with the development of multiple basal cell carcinomas (BCCs) in North America, Europe and Australia (5–7). In Southern Australia HLA DR1 was associated with the development of multiple BCCs at an early age, but not with the development of a single BCC at an early age (7,8). This suggested that different factors are involved in the development of multiple BCCs, possibly factors related to the immune system. Investigations of the immune system of skin cancer patients have yielded conflicting results, possibly because homogeneous groups of patients were not studied. Investigations included

patients with basal cell naevus syndrome (9), xeroderma pigmentosum (10) and cancers of the lip (11), and some studies had patients who had BCC or squamous cell carcinoma (SCC) (10,12,13). The purpose of this study was to investigate cell-mediated immunity in a homogeneous group of patients, those who have had only BCCs removed, and to see if the number of BCCs removed was associated with any abnormality of immunity.

MATERIALS AND METHODS

The research setting was Melbourne, Australia, which is located at 38 degrees south in the temperate zone of the continent. Patients entered into the study were under the age of 60 years, had histologically confirmed BCC(s) removed, had not had another type of skin cancer, were free from internal cancer, chronic diseases such as renal failure, and were not taking immunosuppressant drugs. They were not related and did not have any of the inherited diseases associated with multiple skin cancers, such as the basal cell naevus syndrome or xeroderma pigmentosum.

Two groups of patients were studied. One group consisted of people who had had multiple BCCs (3 or more) removed and were considered to have a high risk of new BCC formation. The other group consisted of patients who had had one BCC removed but had not developed another during a follow-up period of at least 5 years. This group was considered to have a low risk of new BCC formation. The information recorded was age, sex, number of BCCs removed and family history of skin cancer. Cell-mediated immunity was tested by measuring cutaneous delayed hypersensitivity reactions to recall antigens. In addition, lymphocyte counts and subpopulations were measured.

Multitest CMI

Cutaneous delayed hypersensitivity was measured using the Multitest CMI kit (Pasteur-Merieux, Lyon, France). Each kit consisted of seven antigens in standard doses on a plastic applicator. The kit was applied to the inside of the upper arm, a site not exposed to the sun. The site was examined 48 h later and areas of induration were measured. The mean diameter of each indurated area was determined by measuring two diameters at 90 degrees and dividing the sum of these by two. A reaction was positive if the mean diameter was 2 mm or more. The score was zero if there was erythema but no induration. The sum of the mean diameters was written as the patient's sum score.

The Multitest CMI has been evaluated in populations in Europe, North America and Australia (13–15). Normal men have a sum score of 10 or more and normal women have a sum score of 5 or more. No reaction or one slightly positive reaction suggests anergy. In this study scores of 0–3 were considered to indicate anergy.

Lymphocyte counts and lymphocyte subpopulations were measured using standard flow cytometric methods. This technique gave total lymphocyte counts as well as the CD4 (helper cell) and CD8 (suppressor cell) lymphocyte counts. The statistical methods used were: the two sample *t*-test and Mann-Whitney test for two sample comparisons; the chi-squared test with Yates correction for comparison of proportions; analysis of variance (ANOVA) for analysis of the interactions of age, sex, number of BCCs, immunity status (Multitest CMI score), lymphocyte count, and family history.

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RESULTS

Fifty-one patients were studied, 28 (17 M, 11 F) with multiple BCCs and 23 (11 M, 12 F) with one BCC. The average age of the former group was 46.7 years (range 40–58) and that of the latter group was 45.8 years (range 37–57). The low-risk group had been followed for an average of 66 months (range 60–115 months).

The results are summarized in Table I. Only 6 of the multiple BCC patients had Multitest CMI scores in the normal range, and 22 were below normal (15 or more anergic). Twelve of the low-risk patients had scores in the normal range and 11 were below normal (2 anergic). The differences between the two groups were statistically significant: $p < 0.05$ for normal scores, $p < 0.025$ for low scores and $p < 0.01$ for anergic scores (chi-squared test). The scores of the low-risk patients were not significantly different to those of control populations in other Australian studies (16–18).

The cutaneous delayed hypersensitivity tests showed a trend for lower scores in patients with the largest number of skin cancers, and the percentage of anergic patients increased with increasing numbers of skin cancers removed. This trend was statistically significant: $p = 0.04$ (chi-squared test).

The average lymphocyte counts (thousand million cells per litre) for the two groups of patients are set out in Table I. The low-risk patients had: an average CD4 count of 953 (range 572–1,347); a CD8 count of 410 (118–824); and a lymphocyte count of 2012 (1,153–2,871). There were no significant differences between the two groups. A trend was detected for lower CD4 counts to be associated with increasing numbers of BCCs, but it was not statistically significant. Increasing numbers of skin cancers were associated with a greater percentage of anergic patients. This suggested that anergic patients with BCCs are at risk of developing large numbers of skin cancers, and that Multitest CMI might be useful to identify patients with a poor prognosis. The CD4:CD8 ratio for the resistant group was 2.82, but 2.32 for the multiple BCC group. The difference was statistically significant ($p = 0.04$) (*t*-test).

Nine (39.1%) of the resistant group had a first degree relative with histologically confirmed skin cancer, compared to 18 (64.3%) of the multiple BCC group. The difference was not statistically significant ($p = 0.065$, Fisher's exact test).

Table I. Summary of the results

NS—not significant. 27 multiple BCC patients tested.

	High risk of new BCCs <i>n</i> = 28	Low risk of a new BCC <i>n</i> = 23	<i>p</i> value
Multitest CMI			
Normal score	6	12	<0.05
Low score (Anergic)	22 (15)	11 (3)	<0.025 (<0.01)
Lymphocytes*			
CD4	874	953	NS
CD8	481	410	NS
Lymphocyte CD4:CD8	2012 2.32	1976 2.82	$p < 0.05$

*Average counts thousand million cells per litre

DISCUSSION

The study found that cell-mediated immunity was impaired in patients who had multiple BCCs compared to those with a low risk of new BCC formation. Increasing numbers of skin cancers were associated with a greater percentage of anergic patients. This suggested that anergic patients with BCCs are at risk of developing large numbers of skin cancers, and that Multitest CMI might be useful to identify patients with a poor prognosis.

Cutaneous delayed hypersensitivity reactions to recall antigens have been investigated in patients with internal cancers and melanoma, and poor responses to antigens correlate with a poor prognosis (19–22). Testing is useful to determine prognosis in other diseases as well. A recent prospective study has demonstrated that CD4 testing is the most reliable way of predicting the progression of HIV infection to AIDS (23). But there have been problems with administration of antigens and reproduction of results. The Multitest CMI method overcomes these because it is standardized and easy to apply.

Cutaneous delayed hypersensitivity reactions reflect the number and function of CD4 cells (24). However, the average lymphocyte counts were similar in the low-risk and high-risk patients. There was a trend for the average CD4 count to decrease with increasing numbers of BCCs, but this was not statistically significant. The results indicate that Multitest CMI is better than lymphocyte count for predicting which patients will develop large numbers of skin cancers. The DC4:CD8 cell ratio in the low-risk group was significantly higher than in the group with multiple BCCs. The latter group has a worse prognosis, in that these patients are more likely to develop new skin cancers (2). This is similar to the finding by Robinson et al. (25) in North America. These investigators found that patients with a high CD4:CD8 ratio were significantly less likely to develop new BCCs during a follow-up period of 4 years (25). It is not known if a high CD4 cell count protects against new skin cancers, or a low CD8 cell count increases the risk of skin cancer.

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