

SHORT REPORTS

T Lymphocytes with Fc-IgG Receptors in Skin Cancers

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Abstract. The proportion of peripheral blood lymphocytes with receptors for the Fc fragment of IgG (Tg) has been investigated in 25 patients with squamous cell carcinoma of the skin, in 25 patients with basal cell carcinoma of the skin, and in 33 controls. In patients with squamous cell carcinoma the results showed elevated values for Tg lymphocytes and a negative correlation between these T cells and the lymphocyte response to PHA and ConA. In patients with basal cell carcinoma the values for Tg lymphocytes were normal.

Key words: Basal cell carcinoma; Squamous cell carcinoma; T cells with receptors for Fc fragment of IgG (Tg); Blastic response to mitogens (PHA and ConA)

In a large proportion of patients with neoplastic disease the cell-mediated immune response is known to be deficient. This deficiency has been demonstrated both in vivo with non-neoplastic antigens (streptokinase-streptodornase, *Candida albicans*, dinitrochlorobenzene), and in vitro with non-specific mitogens. Investigations carried out recently in man (12) have concentrated on the role of the suppressor lymphocytes in neoplastic diseases.

The literature contains many reports on studies of immunological surveillance in various non-cutaneous tumours, and this contrasts clearly with the paucity of data on purely skin tumours (4, 9). In a previous study (4) in patients with squamous cell carcinoma of the skin we noted reduced numbers of cells forming active E rosettes, a deficiency in the blastic response to mitogens (PHA, ConA and PWM) and a reduced in vivo response to bacterial antigens (antistaphylococcal vaccine and streptokinase-streptodornase).

In the present study we investigated the behaviour of Tg lymphocytes in patients with squamous cell and basal cell carcinomas of the skin, with the aim of establishing correlations between the reduction in the cell-mediated immunity and the percentage of Tg lymphocytes.

MATERIAL AND METHODS

Patients

We studied 50 in-patients of both sexes, ranging in age from 35 to 60 years (mean, 50 years). 25 with squamous cell carcinoma and 25 with basal cell carcinoma of the skin. None of them had been treated with corticosteroids or immunosuppressive drugs in the recent past.

Controls

As controls we used 33 healthy adult men and women (hospital staff), ranging in age from 21 to 60 years (mean, 42 years). None of them were known to have a disease that would affect their immune responsiveness.

Lymphocyte isolation

Some 20-30 ml of venous blood was drawn into a heparinized syringe. The lymphocytes were obtained by centrifugation on a Ficoll-Hypaque (FH) density gradient. Separated lymphocytes were washed three times in Hanks' balanced salt solution (HBSS) and resuspended at optimal concentration in Eagle's MEM-Hepes (Eurobio, Paris) with heat-inactivated 10% fetal calf serum (FCS) at a concentration of 4×10^6 /ml. Phagocytic cells were depleted by addition of carbonyl iron (GAF Inc., New York, N.Y.) to suspension and magnetic removal of macrophages that ingested the iron. Phagocytic cell-depleted lymphoid cells were washed twice in HBSS and resuspended at a concentration of 5×10^6 cells/ml. The cell viability was more than 98%, as demonstrated by trypan blue exclusion test.

Lymphocyte cultures

Lymphocytes were cultured at a concentration of 1×10^6 cells/ml. The following mitogens were used: phytohaemagglutinin (PHA-M Difco; Detroit, Michigan, USA) at a concentration of 0.01 ml/ml and concanavalin A (ConA; Pharmacia, Uppsala, Sweden) at a concentration of 20 μ g/ml. The optimal doses of these mitogens were determined in previous experiments by studies on dose-response curves from lymphocyte cultures from normal donors. Lymphocyte response to mitogens was assayed morphologically.

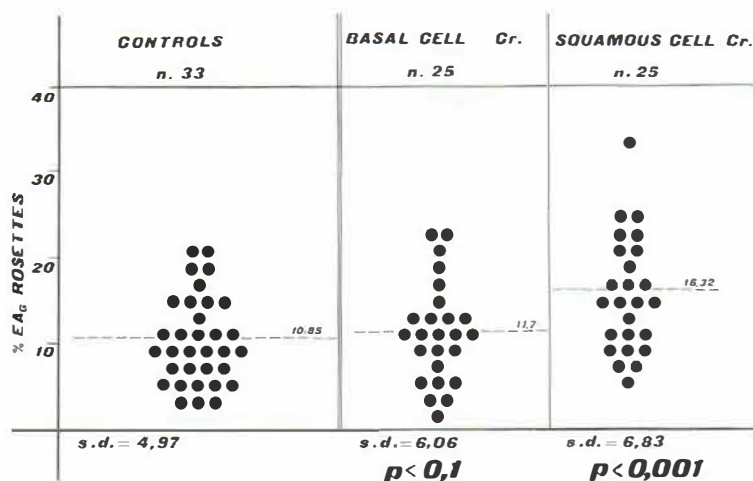


Fig. 1. T lymphocytes with Fc-IgG receptors in skin cancers.

Detection of Tg cells

Purification of T cells. One-millilitre aliquots of phagocytic cell-depleted suspensions were mixed with equal volumes of 1% neuraminidase-treated sheep red blood cells (SRBC). The mixtures were incubated at 37°C for 5 min, centrifuged at 200 g for 5 min and incubated at 4°C for 1 hr. After incubation, the pellets were gently resuspended. The rosetting T cells were then separated from non-rosetting cells on an FH gradient by centrifugation in a temperature-controlled centrifuge at 22°C for 20 min at 400 g. SRBC attached to T lymphocytes were lysed with Tris-buffer containing 0.83% ammonium chloride (pH 7.2). T cells obtained in this way were more than 96% purified, as determined by the rosette formation with SRBC and the lack of cells with readily demonstrable surface immunoglobulin. The viability was greater than 98%, as determined by trypan blue exclusion test. Purified T cells were resuspended in Eagle's MEM-Hepes containing 10% FCS, penicillin 100 units/ml, streptomycin 100 µg/ml, and L-glutamine 2 mM/ml at a concentration of 4×10^6 /ml, and examined for the number of Tg cells.

Determination of T cell subsets. Rabbit IgG anti-OxRBC antibodies were prepared according to Gupta & Good (2).

Preparation of OxRBC-antibody complexes. OxRBC (less than 1 week old) were washed twice in HBSS and resuspended to a concentration of 2%. Equal volumes of 2% OxRBC and IgG anti-OxRBC antibody (subagglutination dilution) were incubated on a rotator at room temperature for 1 hr. Complexes were then washed three times with HBSS and resuspended to a concentration of 1%.

T cells with receptors for IgG (Tg cells). One hundred microlitres of T cell suspension were mixed with 100 µl of OxRBC coated with IgG anti-OxRBC antibody. The mixture was centrifuged at 200 g for 5 min and incubated for 1 hr at 4°C. After incubation, the pellet was resuspended, a drop of trypan blue was added, and 200 lymphocytes were counted for rosette formation.

Statistical evaluation of results

Statistical significance was assessed by value for probability (P) based on Student's t -test.

RESULTS

Tg lymphocytes

No significant difference ($p < 0.1$) could be demonstrated between the basal cell carcinoma group and the controls (Fig. 1).

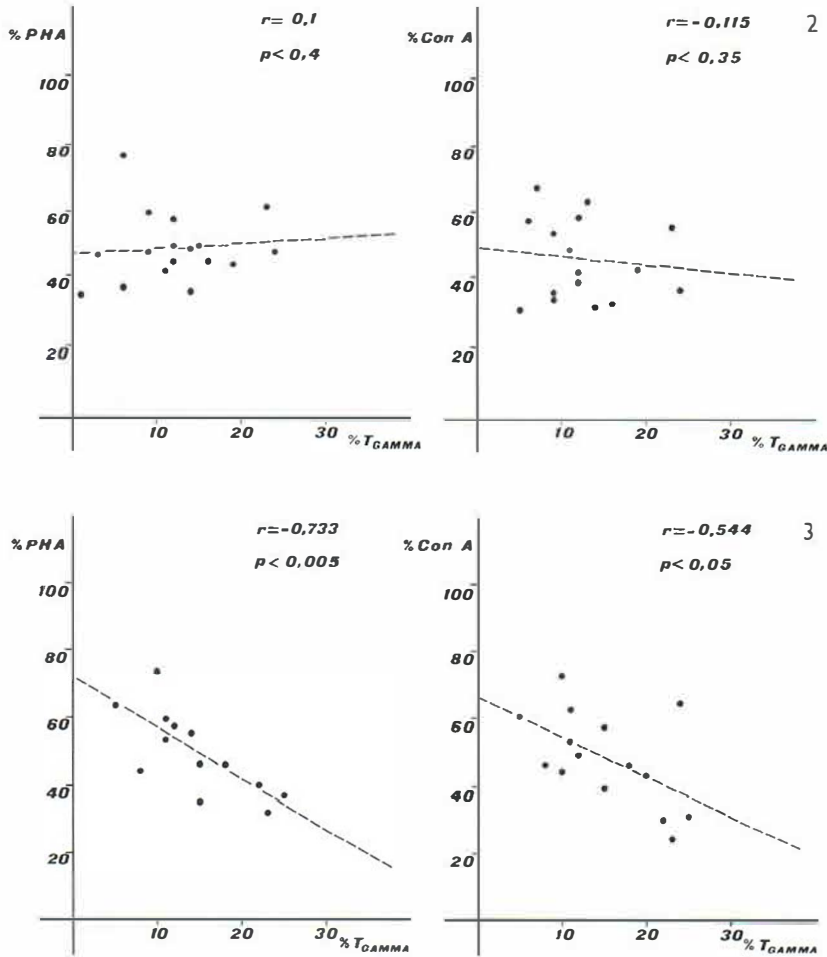
The values obtained in patients with squamous cell carcinoma were significantly higher ($p < 0.001$) than the control values (Fig. 1).

Correlation between percentage of Tg lymphocytes and blastic response to PHA and ConA

In patients with squamous cell carcinoma the values for blastic responses to ConA ($p < 0.05$) and to PHA ($p < 0.005$) were inversely proportional to the percentages of lymphocytes Tg (Fig. 3). No such inverse correlation between the percentage of Tg lymphocytes and the blastic response to PHA ($p < 0.4$) and to ConA ($p < 0.35$) could be demonstrated in patients with basal cell carcinoma (Fig. 2).

DISCUSSION

The immunodepression observed in vitro in patients with various types of lymphoid neoplasia is attributed to the activity of suppressor cells. Increased suppressor activity, affecting the adherent



Figs. 2 and 3. Correlation between the percentage of Tg lymphocytes and the blastic response to PHA and ConA in patients with basal cell carcinoma (Fig. 2) and squamous cell carcinoma (Fig. 3).

cell fraction in particular, has been observed by Zembala (12) in patients with solid malignant lymphoid tumours. However, the same author also noted increased suppressor activity in the lymphocyte T fraction. Moreover, increased numbers of Tg cells with suppressor activity have been reported in Hodgkin's disease (6). Increased numbers of T lymphocytes with receptors for Fc fragments of IgG have been demonstrated also in patients with squamous cell cancers of the buccal cavity (1); the authors concerned admit, however, that the biological function of these cells requires further study. West (11) reported increased values for Tg lymphocytes in patients with malignant tumours, which were associated with reduced percentages of cells forming E rosettes at 29°C. Kishimoto (3) demonstrated a reduced peripheral blood blastic response to PHA in healthy subjects with high per-

centages of Tg and tentatively attributed this reduction to suppressor activity of Tg lymphocytes. In patients with squamous cell carcinoma of the lungs the presence of lymph gland metastases has been attributed to increased numbers of Tg lymphocytes and to increased production of suppressor factors soluble in the lymph glands harbouring the metastases (8).

Our results demonstrated increased percentages of Tg lymphocytes and an inverse correlation between these T cell percentages and the blastic response to PHA and to ConA in patients with squamous cell carcinoma of the skin. In contrast, in patients with basal cell carcinoma of the skin the percentage of Tg lymphocytes was normal. These findings, combined with the well known tendency of squamous cell epithelioma to metastasize, suggest—in agreement with other authors' findings

in other types of tumours (8)—that in the case of squamous cell epithelioma a connection may exist between the increase in suppressor cells and the formation of metastases. This hypothesis finds further support in the blastic response reduction to PHA and to ConA, as recorded by us in carcinoma patients with high peripheral blood levels of Tg.

The results of several studies indicate that, in addition to the suppressor cells, the lymphocyte T fraction with receptors for the Fc fragment of IgG comprises two cell populations, referred to respectively as K (responsible for antibody-dependent cellular cytotoxicity) and NK (to which cell-mediated spontaneous natural cytotoxicity may be attributed (10)). In the light of these data, the Tg lymphocyte increase demonstrated in the peripheral blood of patients with tumours may also be due to increased K and NK lymphocyte fractions. In this latter case, patients with squamous cell carcinoma of the skin would obviously exhibit high levels of lymphocytic cytotoxic activity (7). Furthermore, Tg lymphocytes are known to be less responsive to mitogens (5) and consequently their increased percentage may in fact be responsible for the reduced blastic response to PHA and to ConA, as demonstrated in our patients with high Tg blood levels.

The above immunopathogenetic considerations clearly indicate the need for further studies aimed at elucidating the biological role of T lymphocytes with receptors for the Fc fragment of IgG in patients with skin cancers.

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REFERENCES

1. Fusjibayashi, T., Sato, O. & Itoh, H.: Stage-related, cell-mediated cytotoxicity with effector cell analysis and computation of cytotoxic activity of T cells and non-T cells. *Cell Immunol* 44: 225, 1979.
2. Gupta, S. & Good, R. A.: Subpopulations of human T lymphocytes. II. Effect of thymopoietin, corticosteroids, and irradiation. *Cell Immunol* 34: 10, 1977.
3. Kishimoto, S., Tomino, S., Inomata, K., Kotegawa, S., Saito, T., Koruki, N., Mitsuya, H. & Hisamitsu, S.: Age-related changes in the subsets and functions of human T lymphocytes. *J Immunol* 121: 1773, 1978.
4. Lospalluti, M., Vena, G. A., Angelini, G., D'Ovidio, R. & Meneghini, C. L.: Contributo allo studio

dell'immunità cellulo-mediata nei carcinomi squamocellulari e basocellulari. *Giorn It Derm Vener* 115: 439, 1980.

5. Moretta, L., Ferrarini, M., Mingari, C. M., Moretta, A. & Webb, S. R.: Subpopulations of human T cells identified by receptor for immunoglobulins and mitogen responsiveness. *J Immunol* 117: 2171, 1976.
6. Moretta, L., Santoli, D., Moretta, A., Mingari, C. M., Perlmann, P. & Koprowski, H.: Clinical relevance of subpopulations of human T lymphocytes. In *In vitro Induction and Measurement of Antibody Synthesis in Man* (ed. A. S. Fauci & R. E. Ballieux). Academic Press Inc., New York. In press.
7. Pichler, W. J., Gendelman, F. W. & Nelson, D. L.: Fc receptors on human T lymphocytes. *Cell Immunol* 42: 410, 1979.
8. Sekaly, P., Beaulien, R. & Potvin, C.: Suppressor cells in peripheral blood and regional lymph nodes of patients with bronchogenic carcinoma. Abstract at 4th International Congress of Immunology, Paris 21–26 July 1980.
9. Weimar, V. M., Ceilly, R. I. & Goeken, J. A.: Cell-mediated immunity in patients with basal and squamous cell skin cancers. *J Am Acad Dermatol* 2: 143, 1980.
10. West, W. H., Boozer, R. B. & Heberman, R. B.: Low affinity E-rosette formation by the human K cell. *J Immunol* 120: 90, 1978.
11. West, W. H., Payne, S. M., Weese, J. L. & Heberman, R. B.: Human T lymphocyte subpopulations: correlation between E-rosette-forming affinity and expression of the Fc receptor. *J Immunol* 119: 548, 1977.
12. Zembala, M., Mytar, B., Popiela, T. & Asherson, L.: Depressed in vitro peripheral blood lymphocyte response to mitogens in cancer patients: the role of suppressor cells. *Int J Cancer* 19: 605, 1977.

Cutaneous Reactions to Two Fibrin-derived Peptides

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Abstract. Two previously isolated peptides (Ala-Arg-Pro-Ala-Lys and Ser-Gln-Lcu-Gln-Lys-Val-Pro-Pro-Glu-Trp-Lys) derived from fibrinogen and cleaved off during plasmin-mediated fibrinolysis were investigated regarding their effect on vascular permeability in human skin. Intra-