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Monocyte Fc-IgG Receptors Expression and Soluble Suppressor Factor in Skin Squamous Cell Carcinoma

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The expression of Fc-IgG receptors on mononuclear phagocytes (monocytes/macrophages), as well as the activity of soluble immune suppressor supernatant of T-cell proliferation (SISS-T) factor have been investigated in 23 patients with squamous cell carcinoma (SCC) of the lower lip, including 8 patients with metastases in the regional lymph glands. The results demonstrated a significant increase of the expression of Fc-IgG receptors only on circulating monocytes of patients with metastatic SCC. The suppressor function evaluated by means of the SISS-T factor proved normal in patients with non-metastatic skin SCC and reduced in 8 patients with metastatic SCC. *Key words: Skin tumour; Cellular immunity.* (Received April 19, 1983.)

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The monocyte seems to play an important role in anti-tumour immunity. In fact, a considerable body of research data is available, indicating that cells of the mononuclear phagocyte series may recognize and kill malignant cells, thus exercising an important defence function in the host surveillance against neoplastic diseases (1). The monocyte is known to carry membrane receptors for the Fc portion of immunoglobulin G (2). The expression of these Fc receptors on mononuclear phagocytes may undergo changes in response to diverse stimuli, both in vitro and in vivo (3). In particular, it seems to be markedly increased in peripheral blood monocytes of patients with solid malignant tumours (4).

Recently, increasing interest has been expressed in the identification and properties of a number of soluble factors produced by lymphoid cells participating in the control of immune responses. Rich and Pierce (5) described a soluble immune response suppressor factor present in the supernatant of ConA-activated murine spleen cell culture which inhibits the biosynthesis of immunoglobulins. Williams and Korsmeyer (6) and Kaufman et al. (7) described the suppressor factors present in ConA-activated human lymphocyte supernatants which inhibit the mixed leukocyte reaction. Finally, Greene et al. (8) described in supernatants of ConA-activated human peripheral blood mononuclear cells a suppressor factor which inhibits mitogen- and antigen-induced T-cell proliferation (SISS-T).

There have been relatively few studies on the cells which may play a role in immune surveillance against skin tumours. Our previous studies (9, 10, 11) in patients with squamous cell carcinoma (SCC) of the skin produced certain interesting findings: 1) a deficiency in the blastic response to mitogens PHA, ConA and PWM; 2) elevated levels of T lymphocytes with Fc-IgG receptors (Tγ); 3) a negative correlation between Tγ cells and the lymphocyte response to PHA and ConA; 4) decreased levels of theophylline-sensitive T lymphocytes; 5) a normal suppressor function as evaluated by means of soluble immune response suppressor factor in non-metastatic skin SCC; in contrast, this last suppressor

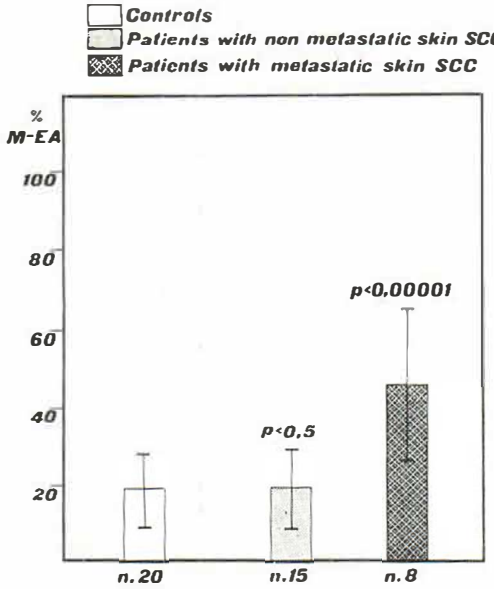

Fig. 1. Expression of Fc-IgG receptors on monocytes in patients with squamous cell carcinoma (SCC) of the skin.

function seemed reduced in metastatic skin cancers. The aim of the present investigation was to contribute further to the study of cell-mediated immunity in patients with SCC of the skin. For this purpose we studied the behaviour of the expression of Fc-IgG receptors on monocytes in peripheral blood cells of patients with skin SCC and correlated the results with those of a study of the suppressor function mediated by the soluble immune suppressor supernatant of T-cell proliferation (SISS-T) factor.

MATERIAL AND METHODS

Patients

We studied 23 patients of both sexes, ranging in age from 35 to 62 years (mean, 50 years), with SCC of the lower lip. Eight of these presented regional metastatic lymph nodes. In all patients there was histological confirmation of the diagnosis. None of them had been treated with corticosteroids, immunosuppressive drugs or radiotherapy in the recent past.

Controls

As controls we used 20 healthy adult men and women (hospital staff), ranging in age from 21 to 62 years (mean, 42 years). None of them were known to have other diseases that would affect their immune responsiveness.

Isolation of mononuclear phagocyte cells

The method used was that of Rhodes (4) modified as follows. Some 20–30 ml of venous blood was drawn into a heparinized syringe. The lymphocytes were obtained by centrifugation on Ficoll-Hypaque (FH) density gradient. Separated mononuclear cells were washed three times in Hanks' balanced salt solution (HBSS) and resuspended at $3–5 \times 10^6$ cells/ml in Eagle's MEM-Hepes (Eurobio, Paris). A 1-ml portion of suspension was placed in 30×10 mm Petri chamber (Flow, U.K.). After incubation at 37°C for 1 hour, the nonadherent cells were removed by washing the monolayer with warm serum-free medium. The presence of 20% fetal calf serum during monocyte adherence is important as it prevents the adherence of lymphocytes. Approximately 95% of the adherent cells at this stage were monocytes as evaluated by non-specific esterase staining according to Yam (12). Sheep erythrocytes bearing a standardized amount of specific antibody were prepared by incubating a 5% suspension of sheep erythrocytes with heat-inactivated rabbit IgG anti-sheep antiserum (purified on a Sephadex G-200 column) at subagglutinating concentration in phosphate-buffered saline. After 30 min at 20°C the cells were washed once and resuspended in HBSS at a concentration of 1%.

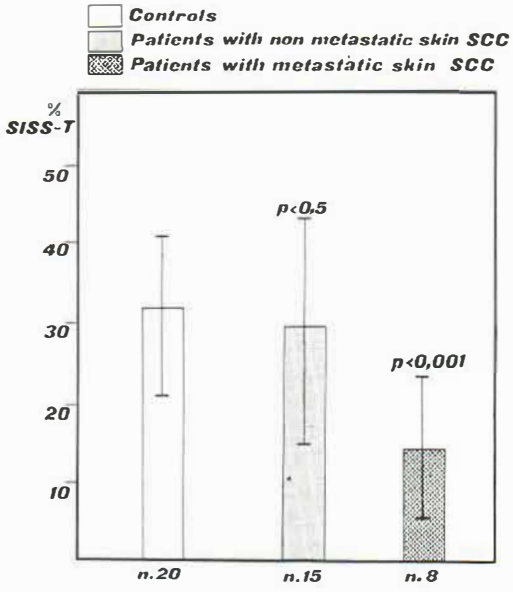

Fig. 2. Suppressor activity mediated by the SISS-T factor in patients with squamous cell carcinoma (SCC) of the skin.

Monocytes with receptors for IgG

One-millilitre aliquots of sensitized erythrocytes were added to each monolayer and alloweed to settle for 1 hour at 20°C. The monolayers were then washed five times with HBSS, fixed with 1% glutaraldehyde and stained with buffered Giemsa. Mounted preparations were examined and cells with five or more attached and/or ingested erythrocytes were scored as rosettes. The percentage of rosette-forming monocytes occurring in a monolayer from a normal donor was compared with that simultaneously occurring in a monolayer from a donor with SCC of the skin.

Preparation of soluble suppressor supernatant of T-cell proliferation (SISS-T) factor

The method used to detect the SISS-T factor was that of Greene et al. (8) modified as follows. Peripheral blood mononuclear cells, obtained by the previously described technique, at a concentration of 3×10^6 cells/ml were incubated at 37°C in 5% Co_2 with Concanavalin A (ConA, Pharmacia, Uppsala, Sweden) at a concentration of 40 μg/ml for SISS-T factor production. After 72 hours, ConA was removed by absorbing the supernatants fluids six times with Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscatway, N.J.). The supernatant thus obtained was then Millipore filter sterilized and used undiluted.

Treatment of cell cultures with SISS-T factor

Supernatants containing SISS-T factor at a concentration of 0.05 ml/ml were added to lymphocyte cultures stimulated by ConA (15 μg/ml) from normal subjects. After incubation at 37°C in 5% CO_2 for 72 hours, lymphocyte responses were assayed morphologically, as radioactive assay can be influenced by the presence of cold thymidine in supernatants containing SISS-T factor (13). All tests were done in triplicate. For each patient the supernatant containing SISS-T factor was added to ConA-stimulated lymphocyte cultures of three different normal subjects in order to avoid false-negative cases and the possibility of 'low responders' (14). The results were accepted if the difference of the various tests was less than 5%.

Determination of suppressor activity

Suppressor activity was calculated as the percentage decrease of the blastic response between the control stimulated cultures and those containing SISS-T factor.

Statistical evaluation of results

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

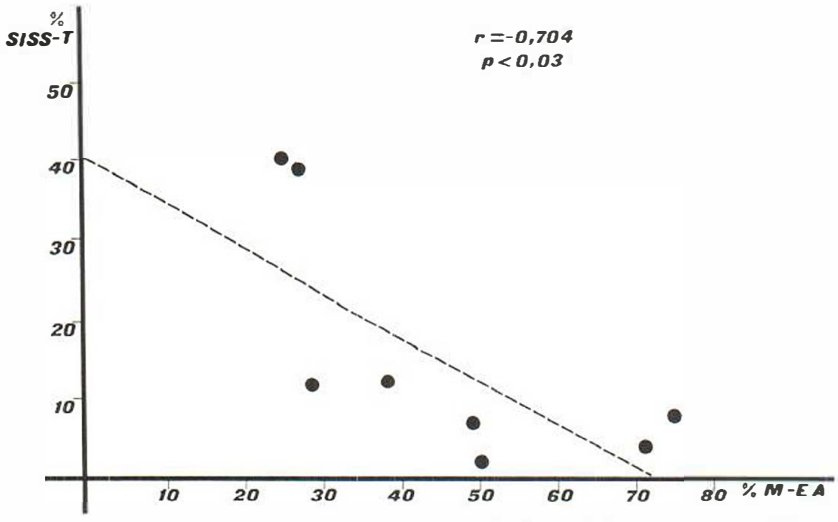

Fig. 3. Negative correlation between monocyte activation and the suppressor activity as evaluated by means of the SISS-T factor in 8 patients with metastatic squamous cell carcinoma of the skin. Each circle represents one individual. *Abscissa:* monocytes (%); *ordinate:* SISS-T factor (%).

RESULTS

Expression of Fc-IgG receptors on monocytes

The monocyte activation values obtained in patients with metastatic SCC were significantly ($p<0.00001$) higher than those in the controls. The converse was true of patients with non-metastatic SCC, in whom the monocyte Fc-IgG receptors expression showed non-significant difference ($p<0.5$) from that in the controls (Fig. 1).

Suppressor activity of the SISS-T factor

The mean values for the SISS-T factor activity recorded for patients with metastatic skin SCC were significantly ($p<0.001$) reduced (Fig. 2). By contrast, no significant differences ($p<0.5$) came to light among the figures recorded for patients with non-metastatic skin cancer vs. the control values.

Correlation between monocyte Fc-IgG receptor expression and the suppressor activity as evaluated by means of the SISS-T factor

As Fig. 3 shows, the values of monocyte Fc-IgG receptor expression of peripheral blood cells in patients with metastatic skin SCC are inversely proportional ($p<0.03$) to the percentage of suppression mediated by the SISS-T factor. In contrast, this inverse correlation between the monocyte Fc-IgG receptor expression and the suppressor activity as evaluated by means of the SISS-T factor could not be demonstrated in patients with non-metastatic skin cancer.

DISCUSSION

The role of the mononuclear phagocyte series in anti-tumour immunity in man is at present a subject of considerable interest. Some recent findings suggest that monocytes may actually be responsible for certain changes in the immunocompetence of patients with cancer.

Berlinger et al. (15) and Zembala et al. (16) showed that monocytes of cancer patients

suppress the blastic response of lymphocytes to mitogens. Furthermore, Rhodes (4, 17) demonstrated an increase in the expression of Fc-IgG receptors on monocytes of patients with visceral carcinomas. Rhodes suggests two possible explanations for these findings. The first interprets monocyte activation, expressed by the increase in the Fc-IgG receptors, as a positive host reaction, which would facilitate macrophage-mediated cytotoxicity and the clearance of immune complexes. In this situation the tumour progress would be favoured by other changes in the host's defence system. The second possibility is that this alteration in the properties of monocyte surface demonstrated in neoplastic diseases might have an inhibitory effect on anti-tumour immunity. This inhibitory effect might manifest itself locally during the initial phase of tumour development and systematically in terminal phase.

Our data obtained in the study reported here emphasize a significant change in monocyte Fc-IgG receptor expression in patients with metastatic skin SCC. This could not be demonstrated in patients with non-metastatic skin SCC, in whom the monocyte Fc-IgG receptor expression showed values statistically no different from those obtained in the controls.

As regards the SISS-T factor in supernatants of ConA-activated peripheral blood lymphocytes of patients with skin SCC, the inhibitory activity of this factor seems to be significantly depressed only in patients with metastatic skin SCC.

Considering our data, it seems that activation of monocytes, expressed by an increase in the Fc-IgG receptors on their membrane, inhibits the activity of ConA-activated suppressor cell, resulting in depression of the SISS-T factor-mediated inhibitory activity. This inhibition would probably occur via prostaglandin (PG) production by activated macrophages. In fact, Passwell et al. (18) showed that addition of Fc fragments and aggregated IgG to monocyte cultures enhances the production of prostaglandin E, and moreover Goodwin (19) demonstrated an inverse relationship between the PG-mediated immunosuppression and the induction of ConA-induced suppressor cell.

As yet it is difficult to interpret the exact meaning of our results, i.e. whether we are faced with immunological changes which may play a protective role or not. Presumably, by following up patients with skin SCC, we shall be able to understand whether monocyte activation, expressed by the increase in the Fc-IgG receptors on their membrane, occurs only when the tumour reaches a certain size or location (e.g. lymph nodes).

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