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# T CELL SUBSETS AND SOLUBLE IMMUNE RESPONSE SUPPRESSOR (SIRS) FACTOR IN SKIN SQUAMOUS CELL CARCINOMA

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*Abstract.* T cell subsets (theophylline-sensitive T lymphocytes and Tγ lymphocytes), and the activity of soluble immune response suppressor (SIRS) factor have been investigated in a total of 53 patients with squamous cell carcinoma (SCC) of the skin. Eight of these had metastatic regional lymph nodes. The results showed decreased values for theophylline-sensitive T lymphocytes and elevated values for Tγ cells. Suppressor function evaluated by means of SIRS factor was normal in 36 patients with non-metastatic skin SCC and decreased in 8 with metastatic skin cancer.

*Key words:* Squamous cell carcinoma; Theophylline-sensitive T lymphocytes; T cells with receptors for Fc fragment of IgG (Tγ); Soluble immune response suppressor (SIRS) factor

Many reports on studies of immunological surveillance in patients with tumours of various types have been published. Recently, the tendency of several authors has been towards the study of the suppressor function of immunocompetent cells and its role in cancer patients (1, 14, 20). In two previous studies (10, 17) carried out in patients with squamous cell carcinoma (SCC) of the skin, we observed: 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) and a negative correlation between Tγ cells and the lymphocyte response to PHA and ConA.

The aim of the present investigation was to contribute further to the study of the suppressor function of peripheral blood lymphocytes in patients with skin SCC. In particular, we investigated the behaviour of theophylline-sensitive T lymphocytes (T-th-sens), which appear to exhibit suppressor capacity (16), and the activity of soluble immune response suppressor (SIRS) factor contained in the supernatants of ConA-stimulated T lymphocyte cultures (7, 15). Moreover, we evaluated the percentage of T cells with receptors for the Fc portion of IgG (Tγ) and compared this latter value with that of T-th-sens lymphocytes.

## MATERIAL AND METHODS

### *Patients*

We studied 53 in-patients of both sexes, ranging in age from 35 to 60 years (mean, 50 years), with squamous cell carcinoma 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 or immunosuppressive drugs in the recent past.

### *Controls*

As controls we used 34 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 $4\times10^6$ cells/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 $5\times10^6$ cells/ml. The cell viability was more than 98%, as demonstrated by trypan blue exclusion test.

### *Detection of theophylline-sensitive T cells*

*Theophylline treatment.* For theophylline treatment, equal volumes of mononuclear cells, prepared by the previously described technique, were mixed with theophylline (Sigma Chemical Comp., Saint Louis, Missouri, USA) at a concentration of $5\times10^{-3}$ M and incubated for 2 hours at 37°C in 5% $CO_2$. The viability of treated cells was assessed by trypan blue dye exclusion.

*E rosette assay.* Rosettes were obtained by mixing in equal parts (0.25 ml) lymphocytes treated with theophylline at a concentration of $4\times10^6$ cells/ml with sheep red blood cells (SRBC) washed three times and resuspended at a concentration of $4\times10^8$ cells/ml.

After incubation at 37°C for 5 min, centrifugation at 200 *g* for 5 min and a final incubation at 4°C overnight, the pellet was gently resuspended and the percentage of

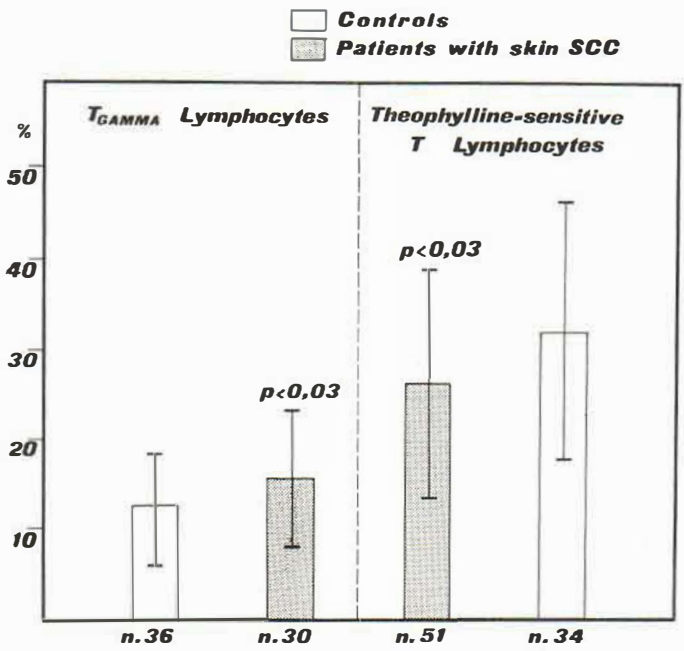


*Fig. 1.* Theophylline-sensitive T lymphocytes and Tγ cells in squamous cell carcinoma of the skin.

rosette-forming lymphocytes (surrounded by 3 or more SRBC) was calculated for 400 consecutive lymphocytes. The determinations were done in duplicate assays for each patient and the results were accepted if the difference between the two tests was less than 5%. Evaluation of theophylline-sensitive T cells was determined by subtracting the percentage of rosettes in the presence of the drug from the percentage of rosettes in its absence; such difference was then expressed as a percentage of the T cell total.

*Detection of Tγ cells*

This procedure has been described in detail in a preceding investigation (17).

*Treatment of lymphocytes with TP-1*

The method of Fiorilli et al. (3) was used. Briefly, the lymphocytes were incubated at a concentration of $4\times10^6$ cells/ml with 50 μg of thymostimulin (TP-1, Istituto Farmacologico Serono, Rome, Italy) at 37°C for 90 min and washed twice. E rosettes were assayed by the previously described thecnique and the percentages of T lymphocytes incubated with TP-1 were compared with the basic values of E rosette-forming cells.

*Preparation of SIRS factor*

The method used to detect the SIRS factor was that of Kaufman et al. (7), modified as follows. Peripheral blood lymphocytes, obtained by the previously described technique, at a concentration of $3\times10^6$ 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 SIRS factor production. After 72 hours, ConA was removed by absorbing the supernatant fluids three times with Sephadex G-50 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.). The supernatant thus obtained was then Millipore filter sterilized and used undiluted.

*Treatment of cell cultures with SIRS factor.* The treatment of cell cultures with SIRS factor was performed *ad modum* Greene et al. (5) with a slight modification. Supernatants containing SIRS 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, lymphocytes responses were assayed morphologically, as radioactive assay can be influenced by the presence of cold thymidine in supernatants containing SIRS factor (12). All tests were done in triplicate. For each patient the supernatant containing SIRS 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" (6). The results were accepted if the difference between the various tests was less than 5%.

*Determination of suppressor activity.* Suppressor activity was calculated as the percentage decrease in the blastic response between the control stimulated cultures and those containing SIRS factor.

*Statistical evaluation of results*

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

## RESULTS

*Theophylline-sensitive T lymphocytes and Tγ cells*

The values for T-th-sens lymphocytes measured in patients with SCC of the skin were significantly

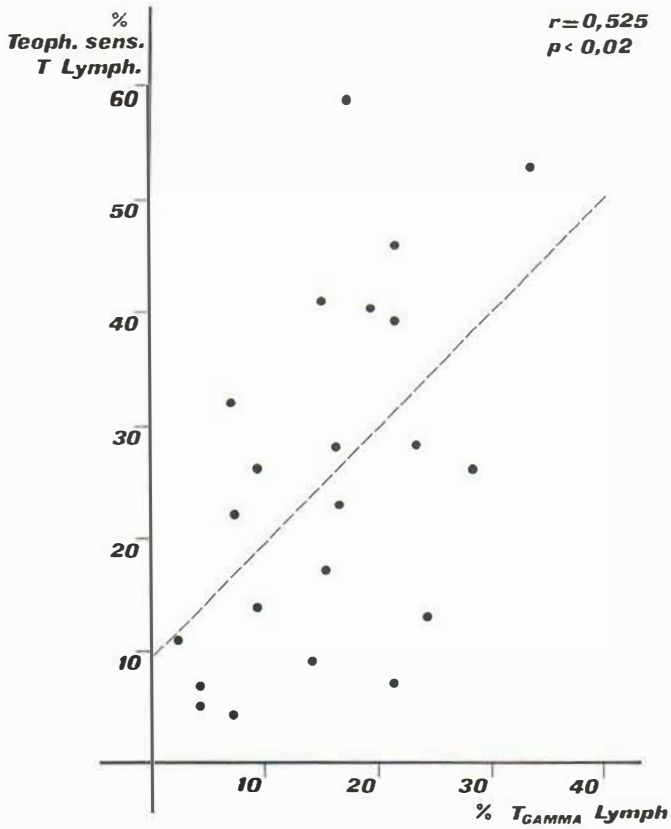


*Fig. 2.* Positive correlation between percentage of theophylline-sensitive T lymphocytes and Tγ cells ($r=0.525$, $p<0.02$). Each circle represents one individual. *Abscissa:* Tγ lymphocytes (%); *ordinate:* theophylline-sensitive T lymphocytes (%).

($p<0.03$) lower than those recorded in controls (Fig. 1). At the same time, as the same figure shows, the results for Tγ lymphocytes proved to be significantly increased ($p<0.03$), compared with controls. This latter observation agrees with our previous findings (17).

*Correlation between the percentages of theophylline-sensitive T lymphocytes and Tγ cells*

Fig. 2 shows that in every one of the patients investigated a positive correlation existed between the percentages of T-th-sens lymphocytes and of Tγ lymphocytes ($p<0.02$). According to Shore et al. (16) this positive correlation would confirm that a majority of the T-th-sens lymphocytes carried receptors for IgG.

*Treatment of lymphocytes with TP-1*

As the data in Table I indicate, incubation of peripheral blood lymphocytes of skin cancer patients with the thymus hormone TP-1 did not increase the E-rosette-forming cell values.

*Suppressor activity of SIRS factor*

The mean values of SIRS factor activity recorded in patients with metastatic skin SCC were significantly ($p<0.001$) reduced (Fig. 3). In contrast, no such significant ($p<0.5$) difference could be demonstrated in patients with non-metastatic skin cancer, compared with controls.

## DISCUSSION

Studies of the behaviour of two lymphocytic subpopulations with suppressor capacity, namely T cells with receptors for the Fc portion of IgG (Tγ) and T-th-sens lymphocytes, demonstrated increased Tγ cell values in patients with skin SCC, thus confirming the findings of our previous study (17). In contrast, the theophylline-sensitive subpopulation, which would seem to correspond to that of T suppressor lymphocytes (16), proved reduced. An interpretation of this reduction in the presence of the Tγ increase, whose biological significance remains uncertain (11, 18), could be based on any of the following considerations.

Table 1. *Values of total E rosettes with and without treatment of the peripheral blood lymphocytes with TP-1 in controls and in patients with squamous cell skin carcinoma*

| Controls | | | Patients | | |
|---|---|---|---|---|---|
| | E rosettes | | | E rosettes | |
| Cases *n* | Basic values | Treatment with TP-1 | Cases *n* | Basic values | Treatment with TP-1 |
| 17 | 61.41±8.65 ($p<0.5$) | 62.29±8.61 | 16 | 67.62±9.0 ($p<0.4$) | 68.81±8.54 |

(*a*) the presence in the bloodstream of immature T lymphocytes which, following in vitro contact with theophylline, become capable of maturing into E-rosette-forming cells and thus to contribute to the population of theophylline-resistent lymphocytes;

(*b*) the presence in the peripheral blood of cells with high cAMP levels, insensitive to theophylline;

(*c*) the lack in the bloodstream, of precursors of T suppressor lymphocytes, forming a part of the T-th-sens lymphocyte population.

The first hypothesis (*a*) can be discarded on the basis of our own results, which indicate that in the peripheral blood of patients with skin SCC the number of full E-rosette-forming cells is not increased by incubation with TP-1. The latter is the thymic hormone which, like theophylline, can induce maturation of immature T lymphocytes into E-rosette-forming cells (3, 9, 13).

The second hypothesis (*b*) is based on the data of a similar study by Ciboddo et al. (2) in a group of patients with pulmonary neoplasms. These authors found that in approximately half the patients the population of T-th-sens lymphocytes was reduced and demonstrated that this reduction might be connected with the in vivo increase of intracellular cAMP sufficient to render the lymphocytes insensitive to theophylline.

The third possibility (*c*) derives from the observation of Shore et al. (16), according to which the population of T-th-sens lymphocytes comprises either suppressor lymphocytes, or their precursors. Furthermore, according to the same authors, a majority of the T-th-sens lymphocytes are Tγ cells. Inasmuch as we noted a positive correlation between the percentages of T-th-sens lymphocytes and Tγ cells, a reduction in the former population might be connected with the absence from the bloodstream, of the precursors of suppressor lymphocytes.

Recently, ever increasing attention has been paid to the identification and properties of a variety of soluble factors produced by lymphoid cells participating in the control of immune responses. One of these biologically active factors is the soluble immune response suppressor (SIRS). Rich & Pierce (15) described a SIRS factor present in the supernatants of the ConA-activated murine spleen cell culture which inhibits the biosynthesis of immunoglobulins. Williams & Korsmeyer (19) and Kaufman

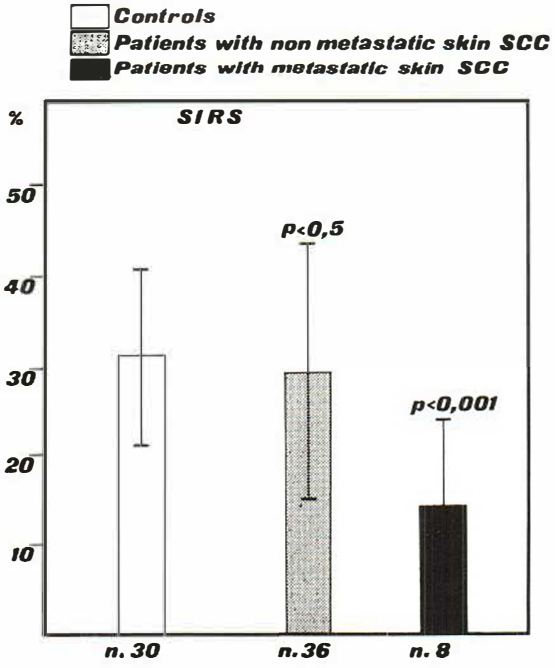


*Fig. 3.* Suppressor activity of SIRS factor in patients with skin squamous cell carcinoma.

et al. (7) described suppressor factors present in ConA-activated human lymphocyte supernatants which inhibit the mixed leukocyte reaction. More recently, Greene et al. (5) described, in supernatants of ConA-activated human peripheral blood mononuclear cells, a suppressor factor that inhibits mitogen- and antigen-induced T cell proliferation.

With regard to SIRS factor in supernatants of ConA-activated peripheral blood lymphocytes of patients with skin SCC, our results have demonstrated that the inhibitory activity of this factor is significantly depressed only in patients with metastatic skin SCC. These preliminary data from our study, which will be a subject of further investigation, agree with the findings of Goodwin (4) who demonstrated that in patients with disseminated malignancies the activity of the ConA-activated suppressor cells is reduced. Since it is also a known fact that the SIRS factor may exhibit its suppressor activity through the macrophages (15) and that the latter are capable, moreover, of modulating the activity of T-suppressor lymphocytes (8), further studies on macrophage functions in patients with skin SCC are in progress.

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