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The control of the contact sensitivity skin reaction: T-suppressor afferent cell blocks the production of antigen-specific T-helper factor

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Summary. Lymph node cells from mice painted with the contact sensitizers picryl chloride or oxazolone produce antigen-specific T-helper factor. This is detected by its ability to increase the contact sensitivity response to the injection of small numbers of haptenized spleen cells into the footpads of naive recipients. The production of this T-helper factor is inhibited by the injection of spleen cells from mice given water-soluble, chemically reactive hapten such as picrylsulphonic (trinitrobenzenesulphonic) acid —an agent which induces unresponsiveness. The cells which inhibit the production of T-helper factor are antigen-specific T-suppressor cells. They are sensitive to cyclophosphamide given before the injection of picrylsulphonic acid, but are unaffected by adult thymectomy. In this respect, they resemble the family of Ts-aff which inhibit the development of contact sensitivity, specific antigen-induced lymph node proliferation and the specific IgG response, and differ from the T-suppressor efferent cell (Ts-eff) which acts at the expression stage of the contact sensitivity reaction. These results are fully compatible with the view that the Ts-aff inhibits the development of contact sensitivity by blocking the production of antigen-specific T-helper factor.

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INTRODUCTION

T suppressor afferent (Ts-aff) lymphocytes are cells, or a family of cells, which are found in the lymphoid tissue of mice immunized with picryl chloride or rendered partially unresponsive by the injection of picrylsulphonic acid (PSA) or haptenized cells (Claman *et al.*, 1980). Their activities include the inhibition of the development of contact sensitivity and of antigen-specific proliferation in the regional lymph nodes, the inhibition of the IgG but not the IgE antibody response, and the inhibition of haptene-specific cytotoxicity (Wood *et al.*, 1977; Thomas, Watkins & Asherson, 1978, 1979a; Tagart, Thomas & Asherson, 1978; Finberg *et al.*, 1979).

The discovery of antigen-specific T-helper cells and antigen-specific T-helper factor in contact sensitivity to picryl chloride (Miller & Butler, 1983; Colizzi *et al.*, 1984) raised the question as to whether the cyclophosphamide-sensitive family of Ts-aff acts on the T-helper cell. Miller & Butler (1983) showed that a T-suppressor cell population, produced by the injection of picrylated cells, blocks the production or action of T-helper cells. They left unresolved two issues. The first is the level of action of the Ts-aff. In particular, does the Ts-aff act by preventing (i) the generation of the T-helper cells or the production of antigen-specific T-helper factor by the T-helper cell, or (ii) the response of the pre-T_{DH} cell to the helper signal? The second issue is whether the T-suppressor afferent cell which

affects the T-helper cell has the characteristics of the classical Ts-aff (Ts_1) which is cyclophosphamide-sensitive but adult thymectomy resistant, or resembles the Ts-eff (Ts_3) which has the opposite properties. This paper shows that the Ts-aff blocks the production of antigen-specific T-helper factor. It also shows that the Ts-aff has the cyclophosphamide sensitivity and resistance to adult thymectomy characteristic of other Ts-aff cells. These findings are fully compatible with the view that the inhibition of the development of contact sensitivity to picryl chloride by Ts-aff is due to inhibition of the production of antigen-specific T-helper factor.

MATERIALS AND METHODS

Production of T-suppressor cells

CBA mice were injected intravenously with 3·5 mg of neutralized picrylsulphonic acid (PSA) on Day 0 and with 3·0 mg on Day 3. Spleen cells were taken on Day 6 or 7. Alternatively 'oxazolone-thioglycollic acid' [8 mg mg *S*-(4-methenyl-2-phenyl-5-oxazolone)thioglycollic acid] was injected twice (Asherson & Zembala, 1981).

Production of antigen-specific T-helper factor (ThF)

Mice were immunized by applying 0·15 ml 5% picryl chloride or 3% oxazolone (4-ethoxymethylene-2-phenyloxazolone) in alcohol to the clipped abdomen, thorax and four paws. The limb girdle lymph nodes were harvested 4 days later. The T cells were purified by nylon wool and the cells cultured at 10^7/ml in RPMI-1640 with 2·5% heat-inactivated fetal calf serum, with added glutamine, penicillin and streptomycin. The supernatant was taken at 24 hr.

Assay of T-helper factor

This method was outlined by Colizzi *et al.* (1984). Spleen cells were picrylated (2×10^7 spleen cells/ml depleted of red cells with Boyle's solution in 1 mM neutralized picrylsulphonic acid in Dulbecco's phosphate-buffered saline for 10 min) or oxazolonated. Oxazolonation was undertaken similarly by adding 20 μl 0·05% oxazolone in ethanol to 2×10^7 spleen cells/ml while 'whirlimixing' in a glass test-tube. The quantity of oxazolone needed varied with the batch, and unduly high concentrations caused cell death. After haptenization and washing three times, the cells were treated with antigen-specific T-helper factor supernatant (10^7 cells/ml, 4°, 1 hr) and washed. A total of 4×10^6 cells were injected into both hind footpads of groups of four to five naive recipients. Five days later, the ears of the mice were painted with 1% picryl chloride or oxazolone, and the increase in ear thickness at 24 hr was expressed in units of 10^3 cm $\pm$ standard deviation.

Adult thymectomy and treatment with cyclophosphamide

Adult thymectomy was performed by a suction method on 5-week-old mice and PSA injected 15–21 days later. Other mice were injected intraperitoneally with 200 mg/kg cyclophosphamide (cy) 2 days before the injection of picrylsulphonic acid.

Treatment with anti-Thy-1.2 serum and complement

Monoclonal anti-Thy-1.2 (OLAC) was used at a dilution of 1/1000 to 5×10^7 cells/ml for 45 min at 4°. The cells were diluted, washed and treated at $2{\cdot}5 \times 10^7$ cells/ml with selected guinea-pig complement (50%) for 45 min at 37° and the same number of live cells injected as in the control.

RESULTS

Inhibition of production of T-helper factor by suppressor cells

Antigen-specific T-helper factor is produced by culturing T cells from the regional lymph node of immune mice. The assay for ThF is based on the fact that small numbers of picrylated cells injected into the footpads fail to induce contact sensitivity unless treated with ThF. In order to investigate the effect of suppressor cells on the production of ThF, mice were injected, at the time of immunization with picryl chloride, with 50 million spleen cells from donors that had received two intravenous injections of picrylsulphonic acid. Figure 1 shows that mice injected with suppressor cells failed to produce ThF (net swelling 0·4 units), while control mice produced ThF (net swelling 2·2 units).

The next experiment investigated the number of suppressor cells needed to block ThF production and the kinetics of its action. Figure 2 shows that 5×10^7 and 10^8 cells block ThF production, while 10^7 cells are inactive. For this reason, 5×10^7 cells were used in all subsequent experiments. Figure 3 shows that the suppressor cells were most active when given on the day of immunization or 1 day later, had less activity when given 1 day before immunization, and were inactive when given 2 or 3 days after immunization.

Specificity of suppressor cells

The following experiment investigated the specificity

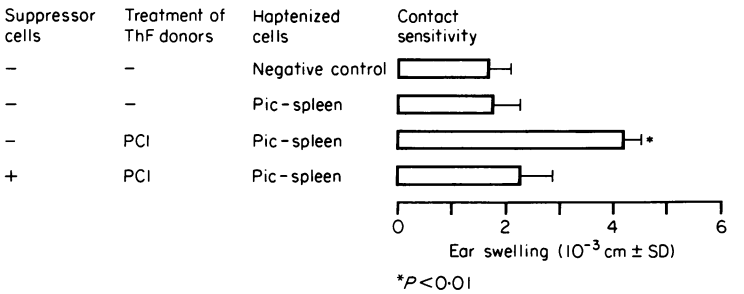

Figure 1. Inhibition of production of T-helper factor by suppressor cells. Mice were painted with picryl chloride and T-helper factor was prepared from the regional lymph node T cells 4 days later. The ThF was tested by incubating normal picrylated spleen cells (Pic-spleen) in it and injecting them into the footpads of groups of five naive recipients. Contact sensitivity was assessed by measuring the increase of ear thickness after challenging on Day 5. The figure shows, in order, the negative control in mice which received no cells, the failure of picrylated spleen cells alone to induce contact sensitivity, and the ability of picrylated spleen cells treated with ThF to induce contact sensitivity. The last line shows that mice which received spleen cells (suppressor cells) from donors injected with picrylsulphonic acid (PSA) failed to make ThF.
*$P < 0{\cdot}01$ in comparison with mice which received picrylated spleen cells alone.

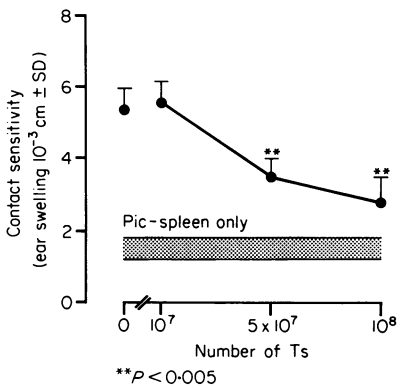

Figure 2. Number of T-suppressor cells needed to inhibit the production of T-helper factor. Mice were injected with various numbers of T-suppressor cells produced by the injection of picrylsulphonic acid. They were then painted with picryl chloride and their ability to produce T-helper factor assessed. Fifty and 100 million cells caused significant depression of T-helper factor production ($P < 0{\cdot}005$).

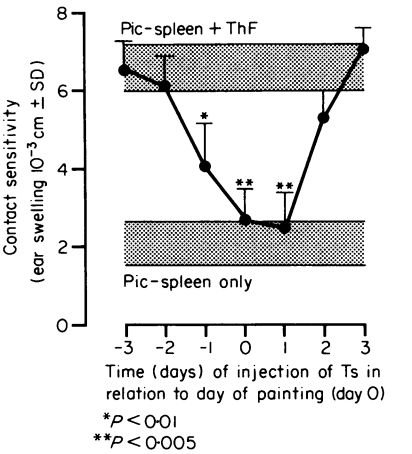

Figure 3. Production of T-helper factor: the effect of injecting suppressor cells at different times in relation to the day of painting. Groups of mice were given 50 million T-suppressor cells from mice injected with picrylsulphonic acid at various times in relation to the day of painting. T-helper factor was prepared on Day 4 and its activity assessed. Statistically significant depression of the production of ThF occurred when the cells were injected on Days $-1, 0, +1$ in relation to the time of skin painting.

of the suppressor cells using suppressor cells to 'oxazolone' induced by the injection of oxazolone-thioglycollic acid. Table 1 confirms the previous finding that oxazolonated cells treated with oxazolone ThF, but not picryl ThF, induce contact sensitivity (Colizzi *et al.*, 1984) and shows that the production of ThF is inhibited by oxazolone-specific, but not by picryl-specific, suppressor cells. The lower part of the table illustrates the specificity of suppressor cells in the picryl system.

In order to exclude the possibility that the suppressor cell generated an inhibitor of the action of ThF, supernatant (which lacks ThF activity) was prepared from immunized mice injected with suppressor cells and mixed 1 : 1 with active ThF. As a control, the active

ThF was diluted with medium. Both mixtures showed ThF activity (4·1 ± 0·25 and 4·4 ± 0·65, respectively).

Characterization of the suppressor cells

It is already known that mice injected with picrylsulphonic acid contain both Ts-aff which are sensitive to cyclophosphamide but resist adult thymectomy, and Ts-eff with reciprocal sensitivities (Thomas *et al.*, 1981a). Figure 4 shows that the cells which suppress the production of ThF are T cells, as indicated by their sensitivity to anti-Thy-1.2 serum and complement, and have the properties of Ts-aff, as judged by their sensitivity to cyclophosphamide given before immunization and their resistance to adult thymectomy.

Table 1. Antigen specificity of suppressor cells in the picryl and oxazolone systems

Suppressor cells	ThF	Contact sensitivity to oxazolone
None	Ox	8·6 ± 0·74*
None	Pic	4·8 ± 0·57
None	None (negative control)	5·1 ± 1·09
Ox	Ox	5·5 ± 0·79
Pic	Ox	8·4 ± 1·37*
		Contact sensitivity to picryl
None	Pic	4·9 ± 0·34**
None	None (negative control)	2·1 ± 0·25
Pic	Pic	2·3 ± 0·57
Ox	Pic	4·0 ± 0·35*

T-helper factor was prepared from mice painted with picryl chloride or oxazolone. Some of these mice were injected before painting with suppressor cells produced by the injection of picrylsulphonic acid or oxazolone-thioglycollic acid.

*$P < 0.01$ in comparison with negative control.

**$P < 0.005$ in comparison with negative control.

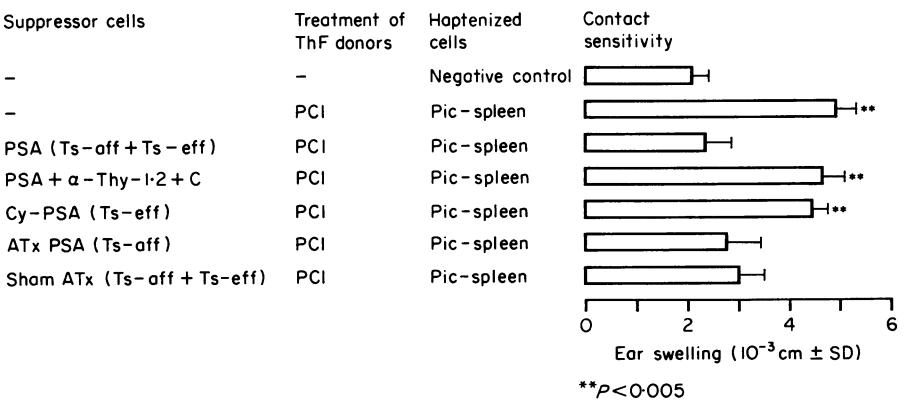

Figure 4. The effect of adult thymectomy and pretreatment with cyclophosphamide on the activity of T-suppressor cells which depress the production of T-helper factor. Mice were injected with cyclophosphamide (cy) 2 days before the injection of picrylsulphonic acid, or subjected to adult thymectomy 3 weeks beforehand or left untreated. The spleen cells were taken on Day 7 and tested for their ability to depress the production of T-helper factor. Some cells were also treated with anti-Thy-1.2 antibody and complement. The variety of suppressor cells injected is shown in parentheses.

DISCUSSION

The present results show that T-suppressor afferent cell(s) which inhibit the development of contact sensitivity also block the production of antigen-specific T-helper factor. This is an extension of the findings of Miller & Butler (1983) that Ts-aff block the production or action of antigen-specific T-helper cells.

Although the injection of picrylsulphonic acid generates distinct Ts-aff and Ts-eff, the cell responsible for blocking the production of T-helper factor is clearly different from the Ts-eff which blocks the passive transfer of contact sensitivity. This is shown by its sensitivity to cyclophosphamide and resistance to adult thymectomy. This characteristic sensitivity is shared by the other Ts-aff cells in the picryl system which affect contact sensitivity, lymphocyte proliferation in the regional lymph nodes, the secondary IgG response and cytotoxicity (Wood *et al.*, 1977; Thomas, Watkins & Asherson, 1979a,b, 1981a; Tagart *et al.*, 1978).

The Ts-aff is antigen-specific as shown in a criss-cross experiment using suppressor cells to picryl and oxazolone groups. Experiment also showed that the Ts-aff prevents the production of antigen-specific T-helper factor (or perhaps the production of the T-helper cell) and does not act by producing an inhibitor of the action of T-helper factor. It is likely that the present Ts-aff affects contact sensitivity by blocking the production of T-helper factor, but it is not yet certain whether the various activities attributed to Ts-aff are due to a single cell or a family of closely related cells. The action on the T-helper cell shown by the present Ts-aff is also shared by Ts-aff cells which affect antibody production (Yamamoto *et al.*, 1977; Kontianinen & Feldmann, 1978; Adorini *et al.*, 1981).

The commonly accepted wisdom is that the Ts-aff acts through the Ts-eff, although there are several different schemas. Thus, Tsurufuji, Benacerraf & Sy (1983) argued that a Ts-aff cell from mice injected with picrylated cells intravenously, made a TsF which augmented (helped) the induction of Ts-eff by antigen (see also Takei, Sumida & Taniguchi, 1983). Another interaction was found by Dorf's group (Minami *et al.*, 1983). They studied a well-characterized set of monoclonal cells in which the Ts-aff (Ts_1) induced an anti-idiotypic Ts_2 in the presence of antigen. The chief action of the Ts_2 was to trigger the Ts_3 (which apparently arises independently) to release antigen-specific TsF (TsF_3).

In contrast, the present set of Ts-aff does not act through the classical Ts-eff (Ts_3). The reason for identifying the classical Ts-eff in the picryl system with Ts_3 is given by Asherson *et al.* (1984). The evidence that the Ts-aff which depresses contact sensitivity and DNA synthesis does not act through the Ts-eff is based on the observation that Ts-aff can be generated in an adult thymectomized mouse and suppress the development of contact sensitivity and antigen-induced DNA synthesis when injected into another adult thymectomized mouse (Thomas *et al.*, 1979a, 1981a). There is also evidence that the Ts-aff which depresses the IgG antibody response does not act through the Ts-eff (Thomas *et al.*, 1979b, 1981b).

In summary, the key features of the present family of Ts-aff are as follows: (i) they only act when given early in the response; (ii) they affect afferent stage phenomena such as lymphocyte proliferation and the production of T-helper factor; (iii) they are sensitive to cyclophosphamide *given before immunization* but are resistant to adult thymectomy; (iv) they do not act through the classical Ts-eff (Ts_3) which is sensitive to adult thymectomy (Asherson *et al.*, 1976). Further experiment will be needed to determine the relationship between the present family of Ts-aff and those described by Minami *et al.* (1983) and other which act through the Ts-eff.

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