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Desensitization *in vitro*: the role of T-suppressor cells, T-suppressor factor and T-acceptor cells in the inhibition of the passive transfer of contact sensitivity to picryl chloride by exposure to antigen *in vitro*

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Summary. This paper investigates desensitization *in vitro*, e.g. the inhibition of the transfer of contact sensitivity to picryl chloride by incubation of the passive transfer population with picrylated spleen cells. It asks whether desensitization is based on the same T-suppressor circuit which is responsible for the inhibition of passive transfer by antigen-specific T-suppressor factor (TsF). In this circuit, the T-suppressor cell which acts at the efferent stage (Ts-eff) makes TsF. This TsF depresses contact sensitivity indirectly by arming a T-acceptor cell (Tacc). The armed Tacc, when exposed to antigen (picrylated spleen cells), liberates a non-specific inhibitor which blocks the transfer of contact sensitivity.

The three elements of this T-suppressor circuit occur in nylon wool-purified T cells prepared from the lymph nodes and spleens of mice four days after immunization with picryl chloride. This population transfers contact sensitivity and can be desensitized *in vitro*. It contains Ts-eff which can be isolated by panning (adherence) on picrylated albumin and detected by their ability to inhibit passive transfer. The 24 hr supernatant of cultures of these cells contains TsF. Finally the population contains Tacc which appear in the spleen 2 days after immunization and virtually disappear by 10 days.

Further experiments demonstrated that the Ts-eff and the Tacc were not merely present but actually required for desensitization *in vitro*. Immune cells depleted of both Ts-eff (by panning on picrylated albumin) and Tacc (by arming with anti-oxazolone TsF and panning on oxazolonated albumin) cannot be desensitized. To restore desensitization both Ts-eff and Tacc must be added back. The Ts-eff were characterized as cyclophosphamide resistant, adult thymectomy sensitive cells (Cy^r, ATx^s), which adhered to antigen and were produced only by specific immunization. The Tacc were characterized as Cy^s, ATx^s cells which adhered to antigen only after arming with antigen-specific T-suppressor factor and were produced after immunization with an unrelated contact sensitizer, 'oxazolone'. It was concluded that desensitization *in vitro* was due to the interaction of two distinct T cells: the T-suppressor cell which acts at the efferent stage of the contact sensitivity reaction and the T-acceptor cell which becomes armed with the specific T-suppressor factor produced by the Ts-eff.

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INTRODUCTION

This paper investigates the relationship between

desensitization of contact sensitivity to antigen *in vitro* and the T-suppressor circuit involving Ts-eff (T-suppressor cell which acts at the efferent or expression stage of the contact sensitivity reaction), T-suppressor factor (TsF) and the T-acceptor cell (Tacc). Uhr & Pappenheimer (1958) described desensitization (inhibition) of the delayed hypersensitivity skin reaction by antigen given at the time of skin testing. Thomas, Smith, Walker & Miller (1981) and others have described desensitization *in vitro*, e.g. the passive transfer of delayed hypersensitivity to the arsanil group is inhibited by incubation of the passive transfer population with arsanilated cells *in vitro*.

The T-suppressor circuit, which this paper argues is the basis of desensitization *in vitro*, starts with the Ts-eff. This is an antigen-directed cell which blocks the passive transfer of contact sensitivity (Asherson, Zembala, Thomas & Perera, 1980). This cell produces an antigen-specific T-suppressor factor (TsF). However, neither the cell nor the factor block passive transfer directly but act indirectly through the macrophage (see Ptak, Zembala, Asherson & Marcinkiewicz, 1981) or the T-acceptor cell. The TsF arms the Tacc which then releases a non-specific inhibitor of passive transfer when exposed to antigen (Zembala, Asherson & Colizzi, 1982a; Zembala, Asherson, James, Stein & Watkins, 1982b; Asherson & Zembala, 1982). The formal resemblance between this circuit and the IgE/mast cell/histamine system is shown in Table 1.

To demonstrate that desensitization *in vitro* is due to this T-suppressor circuit it is necessary to show firstly that the elements of this circuit occur in immune populations which can be desensitized *in vitro* and secondly that these elements are actually needed. This paper indicates that the four day immune lymph node and spleen cells which transfer contact sensitivity to picryl chloride can be desensitization *in vitro* and contain both Ts-eff and Tacc and produce TsF. Moreover the passive transfer population cannot be desensitized after removal of Ts-eff and Tacc. Restoration of desensitization requires the addition back of both Ts-eff and Tacc. It may be concluded that desensitization *in vitro* is due to the occurrence in four day immune populations of a complete suppressor circuit including T suppressor cells with the capacity to make T suppressor factor and T acceptor cells.

MATERIALS AND METHODS

General

See Zembala & Asherson (1980). CBA mice were bred locally and the final recipients used at 6–12 weeks. Thymectomy was undertaken at 5 weeks and mice used 3 weeks later. Cyclophosphamide (150 mg/kg) was given 2 days before immunization to determine the sensitivity of Tacc to its action. The four day immune cells used in all the experiments were prepared

Table 1. The analogy between the suppressor T-cell circuit and the IgE/mast cell/histamine system

Cell producing antigen-specific factor	Antigen-specific factor	Cell armed by antigen-specific factor	'Triggering' antigen	Non-specific product
T-suppressor cell(s) (Ts-eff)	T-suppressor factor(s) (TsF)	T acceptor cell(s) (Tacc)	Haptenized spleen cell	Non-specific inhibitor of contact sensitivity Non-specific inhibitor of DNA synthesis
T-suppressor cell (Ts-eff)	T-suppressor factor (TsF)	Macrophage	Haptenized spleen cell or protein	Non-specific inhibitor of contact sensitivity
B cell	IgE	Mast cell Basophil	Soluble antigen	Histamine Leukotrienes Prostaglandins

This table shows the formal analogy between antigen-specific TsF, which arms the Tacc, which in turn releases non-specific products when exposed to antigen; and IgE which arms mast cells, which in turn release histamine when exposed to antigen. In both cases the antigen-specific factor acts like a mobile receptor conveying its specificity to an acceptor cell. Note that the Tacc requires immunization, but not specific immunization for its production. The Tacc does not appear after adult thymectomy or injection of cyclophosphamide (ATx^s, Cy^s) while the Ts-eff is ATx^s, but unaffected by cyclophosphamide (Cy^r).

by immunization with 5% (w/v) ethanolic picryl chloride or 3% 2-ethoxymethylene-4-phenyloxazolone (oxazolone or ox) both from British Drug Houses.

Preparation of cells and of antigen

T cells were prepared from pooled immune lymph node and spleen cells or unimmunized spleen cells by nylon wool filtration.

Picrylated spleen cells, which served as the antigen used for desensitization, were prepared from normal spleen cells depleted of red cells with Boyle's solution and haptenized with 10 mM picrylsulphonic acid for 10 min following Henry (1980).

Desensitization in vitro

Four day picryl immune nylon T cells ($1{\cdot}5 \times 10^8$) were incubated with a third of their number of picrylated spleen cells (5×10^7/ml final concentration, 30 min, 37°). This ratio was used unless otherwise stated.

As a control, cells were incubated in tissue culture medium. The cells were then centrifuged and one fifth injected into each of five mice. These were challenged immediately on the ears with 1% picryl chloride in olive oil. Contact sensitivity was assessed by the increment of ear thickness at 24 hr in units of 10^{-3} cm $\pm$ SD. The percentage depression of contact sensitivity was given by the means of the following groups using the formula $100 \times$ [(positive − experimental)/(positive − negative)].

Separation of Ts-eff

To purify Ts-eff the T cells (5×10^7/10 ml in 10% inactivated newborn calf serum) were panned on 9 cm bacteriological Petri dishes coated with picrylated bovine serum albumin Fr. 5 (pic-albumin) and 'neutralized' with 50% newborn calf serum. The cells were centrifuged at 40 *g* for 5 min and kept at 37° for 30 min. The plates were then rocked by hand and the non-adherent cells poured off. The adherent cells (Ts-eff) were rinsed twice with medium and finally removed with a silicone rubber bung (Zembala *et al.*, 1982b).

Production of TsF and detection of TsF and Tacc by a two step, indirect assay

In the experiment in Table 4, T-suppressor factor was produced by incubating 4 day nylon T picryl immune cells (10^7/ml, 24 hr, 37°) in supplemented RPMI 1640 with 5% inactivated foetal calf serum. Anti-picryl TsF for testing the kinetics of appearance of Tacc and anti-oxazolone TsF for depleting populations of, and for purifying Tacc were made from mice injected with picrylsulphonic acid or 'oxazolonethioglycollic acid' (Asherson & Zembala, 1980).

TsF was detected in a two step assay: (i) the production of the non-specific inhibitor by arming Tacc with TsF and triggering the release of the non-specific inhibitor by antigen; (ii) the assay of the non-specific inhibitor by the depression of the transfer of contact sensitivity.

The Tacc were 4 day immune cells produced by immunizing mice with oxazolone. They were armed with the presumptive anti-picryl TsF (3×10^7 nylon T cells/ml, 1 hr, 37°). The cells were then diluted, centrifuged, washed once and handled subsequently as in the assay for T-acceptor cells, i.e. the cells were incubated with a third of their number of picrylated spleen cells, centrifuged and resuspended at 4×10^7/ml in 2·5% inactivated foetal calf serum (4 ml) and incubated for 2 hr at 37°. The supernatant (4 ml) was recovered by centrifugation and stored at −20° if necessary. In the second step the presence of non-specific inhibitor was tested by its ability to block the passive transfer by $2{\cdot}5 \times 10^8$ 4 day pooled lymph node and spleen cells incubated in it (45 min, 37°). The cells were then recovered by centrifugation and 4×10^7 injected into each of five recipients. The positive control group received cells incubated in medium only, while the negative control group did not receive cells. The ears were painted immediately afterwards and contact sensitivity assessed at 24 hr.

T-acceptor cells were assayed by arming with anti-picryl TsF, exposing to picrylated spleen cells as a source of antigen and finally assaying for non-specific inhibitor as described above.

Depletion of Ts-eff and Tacc from 4 day picryl immune cells and the restoration of desensitization by the addition of purified cell populations

The 4 day picryl immune T cells were armed with anti-oxazolone TsF and depleted of Tacc by panning on oxazolonated albumin. They were then depleted of Ts-eff (see separation of Ts-eff). The non-adherent cells lacked both Ts-eff and Tacc, while the adherent cell provided a source of Tacc. The depleted population was able to transfer contact sensitivity but could not be desensitized by antigen *in vitro*. The ability of the separated populations to restore desensitization was studied by adding back the cells, incubation with picrylated spleen cells and finally injection into groups of five recipients. See Desensitization *in vitro*.

RESULTS

Desensitization *in vitro*

Four day immune T cells from mice immunized with picryl chloride were incubated with antigen (picrylated spleen cells) *in vitro* for 1 hr and then injected into normal recipients. These were immediately challenged with picryl chloride and contact sensitivity assessed by ear swelling at 24 hr. Table 2 shows that immune cells incubated in medium conveyed contact sensitivity. This was virtually abolished by incubation with antigen (86% inhibition). This effect was not due to masking of the receptors of the passive transfer cells *in vitro* or to the injection of the antigen into the recipient. The evidence for this is that the removal of cells which adhere to picrylated albumin from the passive transfer population had no effect on the transfer of contact sensitivity but prevented desensitization. It was concluded that desensitization *in vitro* required the presence of an antigen-adherent T cell.

Suppressor cell circuit in lymph nodes and spleen of immune mice

It is known that Ts-eff adhere to antigen (Asherson *et al.*, 1980) and the finding that cells with this property are required for desensitization *in vitro* suggested that

Table 2. Desensitization of the passive transfer of contact sensitivity to picryl chloride by exposure to antigen *in vitro*: the requirement for a T cell which adheres to antigen

	Passive transfer cells (T picryl)			
	Unfractionated	Depleted of cells adhering to pic-albumin	Antigen (picrylated spleen cells)	Contact sensitivity
1.	3×10^7	None (positive control)		$4{\cdot}1 \pm 0{\cdot}46$
2.	3×10^7	—	3×10^7	$1{\cdot}7 \pm 0{\cdot}82$ (86%)*
3.	3×10^7	—	1×10^7	$2{\cdot}2 \pm 0{\cdot}26$ (68%)*
4.	—	3×10^7	—	$3{\cdot}7 \pm 0{\cdot}34$ (14%)
5.	—	3×10^7	3×10^7	$3{\cdot}6 \pm 0{\cdot}50$ (18%)
6.	None	None (negative control)		$1{\cdot}3 \pm 0{\cdot}17$

Four day picryl immune T lymph node and spleen cells (passive transfer T cells) were incubated alone or with picrylated (normal) spleen cells as a source of antigen. They were then injected into groups of five recipients. Passive transfer T cells which failed to adhere to picrylated albumin were studied in the same way.

The percentage inhibition of contact sensitivity is shown in brackets.

* $P \leqslant 0{\cdot}001$ as compared with positive control.

Table 3. The population which transfers contact sensitivity contains T-suppressor cells (Ts-eff) which adhere to antigen

Passive transfer cells (picryl)	Cells panned on pic-albumin		Contact sensitivity (increment of ear thickness at 24 hr)
	Adherent	Non-adherent	
5×10^7	None	(positive control)	$4{\cdot}7 \pm 0{\cdot}29$
5×10^7	6×10^6	—	$2{\cdot}2 \pm 0{\cdot}22$ (74%)*
5×10^7	—	6×10^6	$5{\cdot}0 \pm 0{\cdot}62$ (0%)
None	None	(negative control)	$1{\cdot}3 \pm 0{\cdot}17$

Four day picryl immune cells were used as a source of passive transfer cells. They were also used to prepare nylon wool-purified T cells which were panned on picrylated albumin. The adherent cells are presumptive Ts-eff. Passive transfer cells together with the adherent or non-adherent population were then injected into groups of five recipients and contact sensitivity assessed.

$P \leqslant 0{\cdot}002$ as compared with positive control.

a T-cell suppressor circuit might be responsible for this phenomenon. The following experiments investigated whether the key elements of this circuit—Ts-eff, TsF and Tacc—occurred in the immune cell population 4 days after painting with contact sensitizer, i.e. in the population which transfers contact sensitivity and is susceptible to desensitization *in vitro*. In practice nylon wool-purified, pooled lymph node and spleen cells were used.

Four day picryl immune population contains Ts-eff. Mice given picrylsulphonic acid intravenously contain T cells (Ts-eff) which adhere to pic-albumin and block the passive transfer of contact sensitivity (Asherson *et al.*, 1980). The following experiment investigated whether T-suppressor cells with a similar ability to adhere to antigen occurred after painting with picryl chloride, i.e. in the population routinely used to transfer contact sensitivity and to demonstrate desensitization *in vitro*.

Mice were immunized with picryl chloride and nylon wool-purified T cells prepared from pooled lymph node and spleen cells 4 days later. The cells were separated into adherent and non-adherent populations by panning on pic-albumin. Table 3 shows that six million adherent cells (derived from $1{\cdot}8 \times 10^8$ T cells) inhibited transfer by fifty million immune cells (74% inhibition). The same number of non-adherent cells had no effect. It was concluded from the present results in the picryl system and previous results in the oxazolone system (Zembala *et al.*, 1982b) that Ts-eff occur in mice painted with the dose of contact sensitizing agents customarily used to produce contact sensitivity. Ts-eff have previously been demonstrated in mice painted with a supraoptimal dose of fluorodinitrobenzene (Sy, Miller & Claman, 1977).

Four day immune cells produce T-suppressor factor. Zembala *et al.* (1982b) showed that the T-suppressor factor does not act directly on the cell which transfers contact sensitivity but indirectly through the T-acceptor cell. This is the basis for an indirect two step assay for TsF. In the first step Tacc (which require immunization but not specific immunization for their generation) are armed with the anti-picryl TsF to be assayed. When exposed to picryl antigen they release a non-specific inhibitor. In the second step this inhibitor is assayed by its ability to block the passive transfer of contact sensitivity. See Table 1.

In the following experiment 4 day picryl immune T

Table 4. Detection of T-suppressor factor in supernatant of 24 hr culture of 4 day picryl immune cells by indirect assay

	Production of non-specific supernatant	
Tacc cell population incubated in 24 hr supernatant (presumptive TsF)	Antigen added (picrylated spleen cells)	Effect of non-specific supernatant on passive transfer of contact sensitivity to oxazolone
1. Positive control (passive transfer cells incubated in medium)		5·4 ± 0·82
2. Negative control (no cells transferred)		3·2 ± 0·95
3. 4 day cells, picryl super	yes	3·6 ± 1·38 (82%)*
4. 4 day cells, picryl super	no	5·0 ± 0·50 (18%)
5. 4 day picryl cells, ox super	yes	5·0 ± 0·93 (18%)
6. Non-immune cells, picryl super	yes	5·2 ± 0·75 (9%)
7. Non-immune cells, picryl super	no	4·8 ± 0·57 (27%)

Four day immune lymph node and spleen cells from mice immunized with picryl chloride or oxazolone were used to generate 24 hr supernatants containing anti-picryl or anti-oxazolone (presumptive TsF). The TsF was assayed by arming 4 day immune ox cells (Tacc cell population) or normal cells. See materials and methods. The armed Tacc were then exposed to picrylated spleen cells as a source of antigen and the supernatants harvested and tested for their ability to inhibit the passive transfer of contact sensitivity to oxazolone. Lines 3 and 6 show that the supernatant (TsF) from 4 day picryl immune cells contains TsF as judged by the liberation of non-specific inhibitor when Tacc (but not normal spleen cells which lack Tacc) were incubated with antigen corresponding to specificity to the TsF.

* $P < 0{\cdot}05$ as compared with positive control.

Table 5. Time course of appearance of Tacc in the draining lymph nodes and spleen of mice immunized with oxazolone

Ox immune cells (Tacc)		Pic-TsF	Antigen (picrylated spleen cells)	Effect of supernatant on passive transfer of contact sensitivity to picryl chloride
None		Positive control		6·1 ± 0·63
None		Negative control		3·0 ± 1·06
LN	day 2	+	+	6·2 ± 0·84 (0%)
Sp	day 2	+	+	3·7 ± 0·84 (77%)†
LN + Sp	day 3	+	+	3·4 ± 0·48 (87%)†
LN	day 4	+	+	3·7 ± 0·75 (77%)†
Sp	day 4	+	+	4·1 ± 0·65 (65%)*
LN + Sp	day 4	+	+	4·1 ± 0·63 (65%)*
LN + Sp	day 5	+	+	3·5 ± 0·50 (84%)†
LN + Sp	day 10	+	+	5·8 ± 1·90 (10%)

Nylon wool T cells taken at various times after immunization with oxazolone were incubated *in vitro* with anti-pic TsF. The armed Tacc were then exposed to picrylated spleen cells for 2 hr and the supernatants harvested and tested for their ability to inhibit the passive transfer of contact sensitivity to picryl chloride.

* $P < 0{\cdot}01$.

† $P < 0{\cdot}001$.

cells were incubated *in vitro* and the supernatant taken at 24 hr and tested for anti-picryl TsF activity. Table 4 (line 3) shows that TsF was present (82% inhibition of passive transfer). As a control anti-oxazolone TsF was used instead of anti-picryl TsF (line 5), the Tacc was omitted (lines 6 and 7) or the picryl antigen was omitted (line 4). No significant suppression was seen in any of the controls.

Four day immune population contains Tacc. T-acceptor cells bind TsF and release non-specific inhibitor when exposed to antigen (Zembala *et al.*, 1982b). Table 4 (line 6) confirmed previous results which showed that immunization is required to generate Tacc, although *specific* immunization is not necessary (Zembala *et al.*, 1982b). The following experiment investigated the time course of the appearance of Tacc using the indirect assay. Table 5 shows that spleen cells developed Tacc activity 2 days after painting with oxazolone. Pooled lymph node and spleen cells contained Tacc on days 3, 4 and 5 but the activity was virtually absent on day 10.

T-suppressor circuit is required for desensitization *in vitro*

The previous section showed that the elements of the T-suppressor circuit occur in the 4 day picryl immune population which is susceptible to desensitization *in vitro*. This section shows that two distinct cells, one with the properties of Ts-eff and the other with the properties of Tacc, are required for desensitization *in vitro*.

Adherence of cells to antigen. The Ts-eff produced by painting with picryl chloride adhere to pic-albumin. However the Tacc fail to adhere to pic-albumin but adhere to ox albumin after arming with anti-ox TsF (Zembala *et al.*, 1982b). The following experiments investigate whether these two cells were required for desensitization *in vitro*.

Table 6 (lines 1, 2, 3 and 4) illustrates the phenomenon of desensitization *in vitro*. Line 6 shows that the immune population depleted of both Ts-eff and Tacc could not be desensitized. The addition of 4·4 million Ts-eff or 8·8 million Tacc did not restore desensitization. However restoration with both these cells allowed complete desensitization (line 9, 97% inhibition). These results were confirmed in Table 7 (lines 5, 6 and 7).

These experiments do not formally prove that two different suppressor cells are required for desensitization as the results might be explained by the additive effects of the two populations. To resolve this point use was made of the fact that in the T-suppressor circuit the Ts-eff is antigen-specific, while Tacc can be

Table 6. Desensitization of the passive transfer of contact sensitivity to picryl chloride by exposure to antigen *in vitro*: the requirement for two distinct T cells with the properties of Ts-eff and Tacc

Passive transfer cells§ (picryl)	Ts-eff*	Tacc†	Antigen (picrylated spleen cells)	Contact sensitivity to picryl chloride
1. $4{\cdot}6\times10^7$ crude	(positive control)			$5{\cdot}1\pm0{\cdot}44$
2. $4{\cdot}6\times10^7$ crude			$1{\cdot}5\times10^7$	$2{\cdot}0\pm0{\cdot}59$ (84%)¶
3. 3×10^7 T	(positive control)			$5{\cdot}1\pm0{\cdot}44$
4. 3×10^7 T			$1{\cdot}5\times10^7$	$1{\cdot}5\pm0{\cdot}33$ (97%)¶
5. 3×10^7 *depleted*‡	(positive control)			$4{\cdot}6\pm0{\cdot}20$
6. 3×10^7 *depleted*			$1{\cdot}0\times10^7$	$4{\cdot}4\pm0{\cdot}57$ (6%)
7. 3×10^7 *depleted*	$4{\cdot}4\times10^6$		$1{\cdot}0\times10^7$	$4{\cdot}7\pm0{\cdot}58$ (0%)
8. 3×10^7 *depleted*		$8{\cdot}8\times10^6$	$1{\cdot}0\times10^7$	$4{\cdot}2\pm0{\cdot}50$ (12%)
9. 3×10^7 *depleted*	$4{\cdot}4\times10^6$	$8{\cdot}8\times10^6$	$1{\cdot}0\times10^7$	$1{\cdot}5\pm0{\cdot}30$ (97%)¶
10. None (negative control)				$1{\cdot}4\pm0{\cdot}25$

*†‡ The purified Ts-eff (*) and Tacc (†) were obtained by arming 4 day picryl immune T cells with anti-oxazolone TsF. The Tacc and Ts-eff were then purified by panning sequentially on ox albumin and pic albumin. The residual population served as depleted cells (‡).

§ The four day picryl immune 'passive transfer' cells were either unseparated (crude) or nylon wool-purified (T) or depleted of Ts-eff and Tacc (*depleted*).

In lines 7, 8 and 9 the depleted population was 'restored' with Ts-eff or Tacc or both before investigating whether antigen (picrylated spleen cells) caused desensitization *in vitro*.

¶ $P<0{\cdot}001$.

Table 7. Desensitization *in vitro*: the requirement for two distinct T cells one produced by specific immunization and the other produced by both specific and non-specific immunization.

Passive transfer T cells (picryl)	4 day pic immune purified		Four day ox immune (Tacc)§	Antigen (picrylated spleen cells)	Contact sensitivity to picryl chloride
	Ts-eff*	Tacc†			
1. 4×10^7 T	(positive control)				$6{\cdot}7\pm0{\cdot}76$
2. 4×10^7 T				$1{\cdot}4\times10^7$	$3{\cdot}2\pm0{\cdot}84$ (67%)**
3. 4×10^7 *depleted*‡	(positive control)				$6{\cdot}5\pm0{\cdot}40$
4. 4×10^7 *depleted*				$1{\cdot}4\times10^7$	$5{\cdot}9\pm0{\cdot}84$ (12%)
5. 4×10^7 *depleted*	5×10^6	—	—	$1{\cdot}4\times10^7$	$5{\cdot}0\pm0{\cdot}65$ (30%)¶
6. 4×10^7 *depleted*	—	5×10^6	—	$1{\cdot}4\times10^7$	$6{\cdot}0\pm6{\cdot}54$ (10%)
7. 4×10^7 *depleted*	5×10^6	5×10^6	—	$1{\cdot}4\times10^7$	$2{\cdot}7\pm0{\cdot}42$ (76%)††
8. 4×10^7 *depleted*	5×10^6	—	5×10^7	$1{\cdot}4\times10^7$	$2{\cdot}7\pm0{\cdot}41$ (76%)††
9. 4×10^7 *depleted*	—	5×10^6	5×10^7	$1{\cdot}4\times10^7$	$5{\cdot}1\pm0{\cdot}37$ (16%)¶
10. None (negative control)					$1{\cdot}6\pm0{\cdot}37$

Purified Ts-eff and Tacc and nylon T cells depleted of these two populations were prepared as described in the legend to Table 6.

§ Unseparated 4 day, oxazolone immune cells were also used as a source of Tacc.

¶ $P<0{\cdot}01$.

** $P\leqslant0{\cdot}002$.

†† $P\leqslant0{\cdot}001$.

Table 8. Desensitization *in vitro*: the requirement for two distinct T cells with different sensitivities to adult thymectomy and cyclophosphamide only one of which is produced by nonspecific immunization

	Passive transfer T cells (picryl) depleted	4 day immune cells presumptive		Antigen (picrylated spleen cells)	Contact sensitivity to picryl chloride
		Ts-eff*	Tacc†		
1.	3×10^7	(positive control)			$4{\cdot}1 \pm 0{\cdot}24$
2.	3×10^7			1×10^7	$4{\cdot}2 \pm 0{\cdot}43$
3.	3×10^7		ox	1×10^7	$4{\cdot}3 \pm 0{\cdot}29$
4.	3×10^7	Cy picryl	ox		$5{\cdot}7 \pm 0{\cdot}97$
5.	3×10^7	Cy picryl	ox	1×10^7	$2{\cdot}1 \pm 0{\cdot}29$ (78%)‡
6.	3×10^7	Cy picryl	— Cy ox	1×10^7	$4{\cdot}9 \pm 0{\cdot}29$
7.	3×10^7	Cy picryl	— ATx ox	1×10^7	$5{\cdot}0 \pm 0{\cdot}49$
8.	3×10^7	— ATx picryl	ox		$4{\cdot}7 \pm 0{\cdot}68$
9.	3×10^7	— ATx picryl	ox	1×10^7	$4{\cdot}2 \pm 0{\cdot}24$
10.	None	(negative control)			$1{\cdot}1 \pm 0{\cdot}10$

Pic immune T cells were depleted of Ts-eff and Tacc and then restored with unpurified Ts-eff or Tacc or both before investigating whether antigen (picrylated spleen cells) caused desensitization *in vitro*.

* The T cells ($1{\cdot}6 \times 10^7$) from picryl immune mice pretreated with cyclophosphamide served as Ts-eff free of Tacc. They disappeared after adult thymectomy (lines 8 and 9).

† The T cells ($1{\cdot}6 \times 10^7$) from ox immune mice served as Tacc free of anti-picryl Ts-eff. They did not appear after treatment with cyclophosphamide or adult thymectomy (lines 6 and 7).

‡ $P \leqslant 0{\cdot}001$.

produced by immunization with either picryl chloride or oxazolone. Table 7 (lines 5 and 6) shows that five million Ts-eff or five million Tacc had little ability to restore desensitization. However five million Ts-eff together with five million Tacc (produced by painting with picryl chloride and then purified) or fifty million Tacc (produced by painting with oxazolone) restore desensitization (lines 7 and 8). The addition of fifty million Tacc (produced by painting with oxazolone) to five million purified Tacc did not restore desensitization (line 9). It was concluded that Ts-eff and Tacc are distinct populations, both of which are required for desensitization, and that the Tacc but not the Ts-eff are non-specific and occur after immunization with an unrelated antigen.

Sensitivity to cylophosphamide and adult thymectomy. The following experiment shows that the Ts-eff and the Tacc of the suppressor cell circuit and the corresponding cells involved in desensitization have similar requirements for specific or irrelevant immunization and the same sensitivity to cyclophosphamide and adult thymectomy. Table 8 shows that the depleted population cannot be desensitized. Desensitization was restored when unpurified Ts-eff (specifically immunized picryl cells from mice pretreated with cyclophosphamide) together with unpurified Tacc (irrelevantly immunized ozazolone cells) were added (line 5). It also shows that the specific Ts-eff were Cy^r, ATx^s (lines 5 and 9), while the irrelevantly immunized Tacc were Cy^s, ATx^s (lines 6 and 7). Note that adult thymectomy and the injection of cyclophosphamide were undertaken before immunization and hence their effect may be on precursors or on helper cells needed for the appearance of Ts-eff and Tacc.

DISCUSSION

The T-suppressor circuit involving Ts-eff, T-suppressor factor and T-acceptor cells was first recognized using Ts-eff and TsF produced by injecting picrylsulphonic acid or 'oxazolonethioglycollic acid' intravenously (Zembala *et al.*, 1982b). When the Tacc is armed with TsF and then exposed to antigen corresponding in specificity to the TsF, liberation of non-specific inhibitor(s) occurs. These have two actions: on the one hand the non-specific inhibitor blocks the passive transfer of contact sensitivity; on the other hand it inhibits DNA synthesis when added during the first 8 hr of culture of normal lymphocytes with concanavalin A. This effect is due at least in part to the inhibition of the production of interleukin 2. The non-specific inhibitor has no effect on DNA synthesis

when added after the first 8 hr and does not depress cell viability (Malkovsky, Asherson, Chandler, Colizzi, Watkins & Zembala, unpublished). These findings make it unlikely that the non-specific inhibitor produced by the T-suppressor circuit acts by killing T cells indiscriminately.

This paper shows that desensitization *in vitro* is due to the same T-suppressor circuit. In fact 4 days after painting the skin with picryl chloride, the three elements of the circuit occur in the lymphoid population which transfers contact sensitivity and shows desensitization *in vitro*. The Ts-eff was identified by its ability to adhere to antigen. TsF was detected in the supernatant of 4 day immune T cells cultured for 24 hr *in vitro*. The T-acceptor cell appeared in the spleen 2 days after immunization, reached a peak on days 3–5 and disappeared by day 10.

Previous authors have described the Ts-eff and T-auxiliary suppressor cells of the T-cell circuit following skin painting. Sy *et al.* (1977) found Ts-eff in mice painted with a supraoptimal dose of fluorodinitrobenzene which failed to induce contact sensitivity. The Ts-eff were detected by their ability to inhibit passive transfer in a conventional mixing experiment. In the present experiments, the Ts-eff occurred in the same population which transferred contact sensitivity and hence could not be detected in a simple mixing experiment. However they could be demonstrated after their isolation by panning on antigen (picrylated albumin). The auxiliary T-suppressor cell (Ts-aux) of Sy, Miller, Moorhead & Claman (1979) was produced after painting with fluorodinitrobenzene at a dose optimal for the production of contact sensitivity. It is Cy^s, ATx^s and is probably the same as the Tacc required for the action of antigen specific TsF and desensitization *in vitro*.

The finding of a complete suppressor circuit in mice with contact sensitivity to picryl chloride supports the following model of desensitization. The immunization which produces contact sensitivity also generates Ts-eff and Tacc. In the presence of antigen the Ts-eff is stimulated to produce TsF which arms the Tacc. In the continued presence of antigen the armed Tacc releases a non-specific inhibitor of the transfer of contact sensitivity which is the immediate cause of desensitization. However the demonstration of the elements of the suppressor cell circuit only provides circumstantial evidence and formal proof of their importance requires study of the effect of their removal and of adding back Ts-eff and Tacc on desensitization *in vitro*.

In fact lymphoid cells depleted of Ts-eff or both Ts-eff and Tacc cannot be desensitized, and restoration of desensitization requires the addition of two distinct cells. One cell resembles the Ts-eff. It requires specific induction by antigen, is Cy^r, ATx^s and adheres to antigen (pic-albumin). The other cell resembles the Tacc. It requires immunization but not specific immunization for its generation, is Cy^s, ATx^s and only adheres to antigen (ox-albumin) after arming with anti-oxazolone TsF. The fact that both cells are required for desensitization *in vitro* indicates that the Tacc is not already armed with TsF *in vivo* but becomes armed by TsF produced during incubation *in vitro*. Zembala *et al.* (1982b) also noted that the failure of the Tacc in the picryl system to adhere to pic-albumin implied that it was not armed with TsF *in vivo*.

Desensitization *in vitro* was also described in the mouse by Thomas *et al.* (1981). They observed that arsanil-immune passive transfer cells incubated with antigen (arsanilated cells) *in vitro* failed to transfer delayed hypersensitivity. The desensitization showed the phenomenon of specific turn-on of non-specific suppression, i.e. cells from doubly immunized mice (arsanil and oxazolone) failed to transfer contact sensitivity to oxazolone when exposed to arsanilated spleen cells. As in the present system, desensitization required a T cell which was distinct from the cell which transfers contact sensitivity.

It is not clear whether desensitization by immune complexes is also mediated by Tacc. Liew (1975) working in the rat produced desensitization *in vitro* with immune complexes. This might be due to Tacc as these cells have receptors for Fc (Zembala *et al.*, 1982b). The relationship between desensitization *in vitro* with haptenized cells in the mouse and with bovine gammaglobulin in the guinea-pig is unclear. In fact, Liew (1975) suggested that desensitization *in vitro* in the guinea-pig (Asherson & Stone, 1967) was due to immune complexes. This is possible as the passive transfer cells incubated with bovine gammaglobulin were injected into guinea-pigs which received anti-bovine gammaglobulin serum which increases the 24 hr skin reaction.

Desensitization *in vivo* in the guinea-pig may be similar to desensitization *in vitro* in the mouse. However, macrophages as well as Tacc may be important *in vivo*, as macrophages can behave as acceptor cells and release a non-specific inhibitor when armed with TsF and exposed to antigen *in vitro* (Ptak *et al.*, 1981). Uhr & Pappenheimer (1958) described non-specific desensitization when antigen was injected into

immune animals at the time of skin testing. Thestrup-Pedersen, Dwyer & Askenase (1977) suggested that a T-suppressor cell might be responsible, while Parker, Dwyer & Turk (1981) demonstrated that cyclophosphamide, to which Tacc are sensitive, prevented desensitization. The desensitization was mediated by a non-specific serum factor of mol. wt 10,000–45,000 and a non-specific inhibitor occurs in lymphocyte supernatants which also contain migration inhibition factor (Yoshida & Cohen, 1974; Papermaster, Yoshida & Cohen, 1978). It may be relevant that the non-specific inhibitor produced by Tacc has a mol. wt of 30,000–50,000. This limited information suggests that desensitization *in vivo* in the guinea-pig may have certain mechanisms in common with desensitization *in vitro* in the mouse.

What are the functions of the T-suppressor circuit responsible for desensitization? It is known that the local delayed hypersensitivity skin reaction reaches a peak at 24–48 hr and then declines. The cause of the decline is not understood. It is probably not due to simple loss of antigen from the skin test site as cyclophosphamide augments and prolongs the local skin reaction (Turk, Parker & Poulter, 1972). This effect of cyclophosphamide is not due to inhibition of antibody production (Maguire, Faris & Weidanz, 1979). One possibility is that the decline of the local reaction is due to the T-suppressor circuit involving Ts-eff and Tacc and that cyclophosphamide acts by killing or inactivating the T-acceptor cell. The T-suppressor circuit may also be important in viral infections. It is known that delayed hypersensitivity occurs during many viral infections and there is a rapid rise in viral antigen during the first week of infection. It is possible that the T-suppressor circuit which is established by day 4 may limit unwanted immunological damage in the tissues in which the virus is replicating.

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