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T suppressor cells and suppressor factor which act at the efferent stage of the contact sensitivity skin reaction: their production by mice injected with water-soluble, chemically reactive derivatives of oxazolone and picryl chloride

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Summary. The water soluble, chemically reactive thioglycollic acid thioether derivatives of oxazolone and picryl chloride were synthesized and tested for their ability to prevent the development of contact sensitivity. Mice given two injections of these agents showed partial or complete unresponsiveness when subsequently sensitized and challenged with oxazolone and picryl chloride, respectively. This unresponsiveness was associated with T suppressor cells, Ts-eff(cs), which blocked the efferent stage of the contact sensitivity reaction, i.e. the passive transfer of contact sensitivity. These Ts-eff (cs) were entirely specific when tested with the corresponding antigen. However, the suppression which they caused had a non-specific final common pathway. Cells from mice injected with the oxazolone and picryl thioethers and painted with the corresponding contact sensitizer produced a suppressor factor *in vitro*. This factor specifically blocked passive transfer by immune cells incubated in it. It also armed macrophages which then caused suppression. These macrophages were most effective when injected intraperitoneally. The suppressor factor had a molecular weight between 30,000 and 100,000 and the α-oxazolone factor was absorbed by oxazolone-albumin Sepharose and could be eluted with oxazolone-ε-aminocaproic acid. It was also absorbed by concanavalin-A-sepharose and could be eluted with α-methylmannoside. It is proposed that the ability of water soluble, chemically reactive haptenes to evoke a Ts-eff (cs) population may be relevent to the rarity of severe drug reactions following the injection of chemically reactive drugs.

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INTRODUCTION

It is possible to distinguish between T suppressor cells which act at the afferent stage of the immune response, Ts-aff, and those which act at the efferent stage, Ts-eff (Miller, Sy & Claman, 1978a; Asherson, Zembala, Thomas & Perera, 1980). Mice painted on the skin with contact sensitizers, such as picryl chloride and oxazolone (2-ethoxymethylene-4-phenyloxazolone), produce suppressor cells which act when injected early in the immune response. The injection of these Ts-aff into normal mice depresses the lymph node DNA response, the IgG antibody response and, under special circumstances, the generation of cytotoxic T cells which otherwise follow exposure to contact sensitizer.

In contrast, mice injected intravenously with the water-soluble, chemically reactive derivative of picryl

chloride, picryl sulphonic acid, produce both Ts-aff and Ts-eff. The Ts-aff depresses both the lymph node DNA response and the contact sensitivity reaction (Thomas, Watkins & Asherson, 1979, 1980). The Ts-eff blocks the passive transfer of contact sensitivity but has no effect when given at the time of immunization (Thomas *et al.*, 1980). Painting with supraoptimal doses of contact sensitizer also produces Ts-eff (Sy, Miller, Moorhead & Claman, 1979). Populations containing Ts-eff produce a soluble factor *in vitro* which acts, at least in part, through macrophages and suppresses the efferent stage of contact sensitivity (Ptak, Zembala, Hanczakowska-Recklicka & Asherson, 1978).

One of the difficulties in studying Ts-eff produced by picryl sulphonic acid is the lack of another compound with different antigenic specificity which produces these cells. For instance, dinitrobenzene sulphonic acid produces Ts-aff which depress DNA synthesis but not Ts-eff. In fact, it is likely that a few Ts-eff are produced as this cell population produces a T suppressor factor when cultured *in vitro* (Moorhead, 1977). This paper describes a deliberate search for compounds which might generate Ts-eff. Our working hypothesis is that the essential features of picryl sulphonic acid is its chemical reactivity, its water solubility and the feasibility of injecting large quantities intravenously. For this reason the chemically reactive, water soluble picryl and oxazolone thioethers of thioglycollic acid were prepared. This paper shows that mice are made partially unresponsive by the injection of these thioethers and only develop limited contact sensitivity after immunization with the corresponding contact sensitizer. This unresponsiveness is associated with the presence of T suppressor cells, Ts-eff (cs), which block the passive transfer of contact sensitivity. This cell population produces specific suppressor factor(s) which inhibits the passive transfer of contact sensitivity and arms macrophages which then cause suppression.

MATERIALS AND METHODS

Mice

CBA mice, 8–12 weeks old, bred at the Clinical Research Centre or the Institute of Paediatrics, were used.

Antigens

Picryl chloride was purchased from B.D.H. Poole, England or from Fluka and 2-ethoxymethylene-4-phenyloxazolone (oxazolone or ox) from B.D.H. The thioethers of picryl chloride and oxazolone with thioglycollic acid (Pic-TGA and Ox-TGA) were prepared following Cornforth (1949). In more recent preparations, dimethylformamide was used instead of pyridine as a solvent. Oxazolone (2·5 g) was dissolved in a small volume (*ca* 5 ml) dimethylformamide and 1·1 mole-equivalents thioglycollic acid added. The solution was left overnight in the dark and turned red. The crude thioether was precipitated, as an oil, in 0·1 M HCl at 4° for 3 h. The oil was washed first in dilute HCl and then in water before solubilizing in 4 ml tetrahydrofuran. This precipitation and washing was repeated. Finally the red oil was dissolved in aqueous $NaHCO_3$ and brought to pH 7·5. Impurities were removed by extracting three times with chloroform. The yellow solution containing the sodium salt of the thioether was then lyophilized. The picryl thioether was prepared using recrystallized picryl chloride. It was dissolved in pyridine, then precipitated by excess petroleum ether, redissolved and reprecipitated and finally dissolved in aqueous $NaHCO_3$ as above. Some preparations of the oxazolone thioether become insoluble in water after 6 months.

Treatment of mice with antigens

For producing unresponsiveness, 4 mg Ox-TGA or Pic-TGA was injected intravenously on days 0 and 3. Suppressor cells were obtained in the same way using 10 mg and 5 mg, respectively, and harvested 7 days after the first injection.

'Passive transfer' (immune) cells were produced by painting mice with 0·15 ml 3% oxazolone or 5% picryl chloride and taking the regional shoulder girdle and inguinal lymph node and spleen cells on day four.

Production of suppressor factor

Spleen cells and lymph node cells were taken from mice treated to produce suppressor cells and then painted on the sixth day with the corresponding contact sensitizer. They were cultured in Eagle's minimal essential medium (GIBCO) with added glutamine, penicillin and streptomycin and 5% heat inactivated foetal calf serum (Difco) at 10^7/ml for 48 h. The cells were spun down, the supernatant centrifuged at 800 ***g*** and then at 8000 ***g*** for 30 min and stored at −30°.

Adsorption and elution of factor and Amicon membrane filtration

See Asherson, Zembala & Noworolski (1978).

Arming of macrophages
See Zembala & Asherson (1974).

Contact sensitivity
Mice were challenged with 1% oxazolone or 1% picryl chloride on both sides of both ears. The increase in thickness at 24 hours was measured with an engineers' micrometer and expressed in units of 10^{-3} cm (10 μm) $\pm$ standard deviation. The 95% confidence limit was chosen for statistical significance in a two-tailed Student's t test. There were usually five mice in each recipient group.

Arrival of labelled cells
The technique of Vadas, Miller, Gamble & Whitelaw (1975) was used.

RESULTS

Production of unresponsiveness by oxazolone-thioglycollic acid and picrylthioglycollic acid

Mice were injected twice with oxazolone-thioglycollic acid (Ox-TGA) or picryl-TGA (Pic-TGA) on day 0 and 3. Twelve days later, they were sensitized by painting oxazolone on the skin. Contact sensitivity was assessed after a further 5 days. Table 1 shows that Ox-TGA abolished the contact sensitivity response to oxazolone. Pic-TGA had a smaller but definite effect. In a second experiment, Pic-TGA diminished the response to picryl chloride while Ox-TGA had no effect. The Vadas technique, which is based on the arrival of labelled cells, gave a similar result. In fact, the magnitude of the suppression varied without obvious cause and, in a third experiment, 4 mg Ox-TGA only reduced the contact sentitivity response to oxazolone by 37%. Log_{10} dilutions from 400 μg to 400 ng had no effect. It was concluded that Ox-TGA and Pic-TGA caused specific immunological unresponsiveness although there was a variable non-specific effect.

Action of suppressor cells at the efferent stage of the contact sensitivity reaction and their immunological specificity

Suppressor cells were harvested seven days after the injection of Ox-TGA or Pic-TGA and their action tested in a 'mixture experiment'. Suppressor cells, and immune cells, which transfer contact sensitivity were injected into normal mice. These recipients were challenged immediately afterwards and contact sensitivity assessed 24 h later. Table 2 shows that 'Ox-TGA suppressor cells' depressed passive transfer of contact sensitivity to oxazolone by 70%, while 'Pic-TGA cells' had no effect. Similarly, 'Pic-TGA' cells depressed the passive transfer of contact sensitivity to picryl chloride by 100%, while 'Ox-TGA' cells had no effect. It was concluded that the suppressor cells were immunologically specific and blocked the efferent stage of the contact sensitivity skin reaction.

Table 2 also shows the effect of 'Ox-TGA suppressor cells' on passive transfer of contact sensitivity to picryl chloride when the recipients are challenged with a mixture of oxazolone (corresponding to the suppressor cells) and picryl chloride (corresponding to the

Table 1. Depression of contact sensitivity by oxazolone-thioglycollic acid and picryl-thioglycollic acid

	Thioether injected intravenously	Contact sensitizer painted on skin	Contact sensitivity at 24 h	Cell arrival
Exp. 1	None	Oxazolone (positive control)	17·2 $\pm$ 1·81	
	None	None ((negative control)	3·2 $\pm$ 1·35	
	Ox-TGA	Oxazolone	4·7 $\pm$ 1·55	
	Pic-TGA	Oxazolone	11·5 $\pm$ 2·8	
Exp. 2	None	Picryl chl. (positive control)	13·1 $\pm$ 3·30	3·01 $\pm$ 0·47
	None	None (negative control)	2·9 $\pm$ 0·96	1·67 $\pm$ 0·32
	Pic-TGA	Picryl chloride	8·4 $\pm$ 1·52	2·45 $\pm$ 0·26
	Ox-TGA	Picryl chloride	13·1 $\pm$ 2·45	3·36 $\pm$ 0·69

Mice were injected with 4 mg Ox-TGA or Pic-TGA on days 0 and 3, sensitized on day 12 and contact sensitivity measured on day 17. In Exp. 2, the arrival of the animal's own cells labelled with [^{125}I]-iododeoxyuridine at the ears, was measured and expressed as the ratio of the arrival at the painted and unpainted ears.

Table 2. Specificity of the depression of the passive transfer of contact sensitivity by suppressor cells from mice given oxazolone-thioglycollic acid or picryl-thioglycollic acid

Suppressor cells transferred	Passive transfer cells transferred	Mice challenged with	Contact sensitivity at	
			24 h	48 h
None	4 day Ox	Oxazolone	4·3 ± 1·24	5·2 ± 1·02
None	None (negative control)		1·3 ± 0·46	1·4 ± 0·38
Ox-TGA	4 day Ox		2·2 ± 0·53 (70%)	2·3 ± 0·50 (76%)
Pic-TGA	4 day Ox		3·9 ± 0·49 (13%)	5·2 ± 1·04 (0%)
None	4 day Pic	Picryl chloride	3·2 ± 0·24	4·1 ± 0·30
None	None (negative control)		1·4 ± 0·52	1·5 ± 0·43
Pic-TGA	4 day Pic		1·3 ± 0·36 (100%)*	1·8 ± 0·32 (88%)
Ox-TGA	4 day Pic		3·2 ± 0·24 (0%)	4·1 ± 0·30 (0%)
None	4 day Pic	Ox + picryl chloride	6·4 ± 1·20	8·2 ± 0·89
None	None (negative control)		2·8 ± 0·14	3·3 ± 0·35
Ox-TGA	4 day Pic		3·7 ± 0·78 (75%)*	6·3 ± 1·00 (39%)
None	4 day Ox		6·6 ± 1·42	8·4 ± 0·97
Pic-TGA	4 day Ox		5·2 ± 0·76 (37%)	8·1 ± 0·35 (46%)

Donor mice were given two injections of oxazolone thioglycollic acid (Ox-TGA) or picryl thioglycollic acid (Pic-TGA) and the spleen cells harvested 7 days after the first injection. $3{\cdot}6 \times 10^7$ viable cells were transferred to groups of five recipients which also received 4×10^7 mixed lymph node and spleen cells from mice 4 days after immunization with oxazolone (Ox) or picryl chloride. The recipients were challenged shortly afterwards and contact sensitivity measured at 24 h. The percentage depression of contact sensitivity is shown in brackets. Note that the phenomenon is specific when mice are challenged with a single antigen, but that the specific activation of a non-specific depression is seen when mice are challenged with a mixture of oxazolone and picryl chloride.

* Statistically significant depression.

Table 3. Class of suppressor cell in mice injected with oxazolone thioglycollic acid

Suppressor cells transferred	Immune cells transferred	Contact sensitivity at 24 h
None	4 day Ox (positive control)	5·1 ± 0·95
None	None (negative control)	1·6 ± 0·39
Ox-TGA	4 day Ox	2·2 ± 0·41 (83%)*
Ox-TGA + complement	4 day Ox	1·5 ± 1·46 (100%)*
Ox-TGA + α-Thy-1·2 + complement	4 day Ox	4·7 ± 1·13 (14%)
Ox-TGA nylon filtered	4 day Ox	2·2 ± 0·19 (83%)*
ox-TGA nylon adherent + complement	4 day Ox	2·9 ± 1·03 (63%)*
Ox-TGA nylon adherent + α-Thy-1·2 + complement	4 day Ox	4·4 ± 1·41 (20%)
Ox-TGA nylon adherent and α-Ig adherent	4 day Ox	4·8 ± 1·17 (8%)

Groups of mice were injected wtih suppressor cells (3×10^7 mixed lymph nodes and spleen cells from mice 7 days after injection of Ox-TGA) and with 'passive transfer' cells (4×10^7 mixed lymph node and spleen cells) from mice painted with oxazolone 4 days beforehand. The mice were challenged shortly after transfer and contact sensitivity measured at 24 h. The percentage depression of contact sensitivity caused by the suppressor cells is shown in parentheses. Note that the suppressor cells are Thy-1·2 positive and both nylon wool passing and adherent. B cells prepared from the nylon adherent cells by adherence to anti-mouse Ig plates were inactive. There were five mice in each group except the penultimate (3) and the last group (2).

*Statistically significant.

Table 4. Specificity of the depression of the passive transfer of contact sensitivity by incubating cells in α-oxazolone and α-picryl suppressor supernatants

'Passive transfer' cells			Contact sensitivity at	
Immunized against	Incubated in		24 h	48 h
Ox	Eagle's medium	(positive control)	4·1 ± 0·14	4·9 ± 0·83
	No cells transferred	(negative control)	1·3 ± 0·76	1·7 ± 0·50
Ox	α-oxazolone suppressor factor		1·3 ± 0·52 (100%)*	2·0 ± 0·48 (91%)*
Ox	α-picryl suppressor factor		4·3 ± 1·52 (<0%)	5·3 ± 0·87 (<0%)
Pic	Eagle's medium	(positive control)	4·4 ± 1·03	5·2 ± 0·87
	No cells transferred	(negative control)	1·5 ± 0·50	1·9 ± 0·52
Pic	α-picryl suppressor factor		1·9 ± 0·64 (86%)*	2·8 ± 0·50 (73%)*
Pic	α-oxazolone suppressor factor		5·3 ± 0·47 (<0%)	5·7 ± 0·50 (<0%)

'Passive transfer' cells i.e. mixed lymph node and spleen cells were incubated in suppressor factor at a concentration of $2·5 \times 10^7$/ml. Each recipient was given 4×10^7. The percentage depression of contact sensitivity is shown in parentheses.

Table 5. Chemical definition of α-oxazolone suppressor factor

'passive transfer' cells incubated in	Contact sensitivity at 24 h	Contact sensitivity at 48 h
Eagle's medium (positive control)	4·6 ± 0·79	6·1 ± 0·93
No cells transferred (negative control)	1·0 ± 0·34	1·8 ± 0·23
Suppressor supernatant	1·8 ± 0·50 (77%)	2·2 ± 0·21 (91%)
Ox-EACA eluate from Ox-BSA sepharose	2·0 ± 0·51 (72%)	2·6 ± 0·27 (81%)
Control eluate*	5·0 ± 0·76 (<0%)	6·4 ± 1·19 (<0%)
α-methylmannoside eluate from Con-A-sepharose	2·6 ± 0·91 (55%)	3·4 ± 0·81 (63%)
lactose eluate from Con-A-sepharose	5·2 ± 1·39 (<2%)	5·9 ± 1·43 (5%)
Supernatant absorbed (×1) with Ox-BSA sepharose	3·1 ± 0·98 (42%)	3·8 ± 1·44 (53%)
Supernatant absorbed (×2) with Ox-BSA sepharose	3·7 ± 0·76 (25%)	4·6 ± 1·75 (35%)
Supernatant absorbed with Con-A-sepharose	3·5 ± 0·61 (30%)	3·9 ± 0·74 (51%)

* Suppressor supernatant which had been absorbed once with Ox-BSA-sepharose and had little activity (line 8) was reabsorbed and the beads eluted. $5·6 \times 10^7$ mixed lymph node and spleen cells were transferred.

The figures in parentheses show the percentage depression.

passive transfer cells). Under these circumstances, contact sensitivity to picryl chloride is depressed by 75% at 24 h. However, this effect is much less at 48 h. This phenomenon of the specific activation of a non-specific suppression was not seen when 'Pic-TGA' suppressor cells and 'Ox passive transfer cells' were used. It was concluded that at least part of the action of these suppressor cells might be explained by the liberation of a non-specific inhibitory mediator following exposure to specific antigen.

The production of T suppressor cells by oxazolone-thioglycollic acid

Cells were harvested 7 days after the injection of Ox-TGA and their suppressor activity tested in a 'mixture experiment'. Suppressor cells, and immune cells which transfer contact senstivity, were injected into normal mice. Contact sensitivity was assessed 24 h later. Table 3 shows that 'Ox-TGA' cells depressed the passive transfer of contact senstiivity by 83%. This depression was virtually abolished by treatment with anti-Thyl-2

Table 6. Inhibition of passive transfer by α-oxazolone suppressor factor and by peritoneal exudate cells armed with suppressor factor

Treatment of passive transfer cells	Peritoneal exudate cells injected	Contact sensitivity at 24 h	Contact sensitivity at 48 h
No treatment	(Positive control)	5·2±0·89	5·8±0·77
No cell transferred	(Negative control)	1·4±0·43	1·3±0·49
Incubated in suppressor factor		2·2±0·55 (80%)	2·0±0·46 (84%)
No treatment	PEC incubated in SF iv	3·1±0·42 (55%)	2·7±1·02 (69%)
No treatment	PEC incubated in SF ip	1·9±0·54 (87%)	2·3±0·80 (78%)

'Passive transfer' cells (6×10^7 mixed lymph node and spleen cells taken 4 days after painting with oxazolone) were incubated in 5 ml suppressor factor (SF) for 1 hr, washed and then injected. Peritoneal exudate cells ($1{\cdot}5 \times 10^7$) were similarly incubated in 5 ml suppressor factor and injected intravenously (i.v.) or intraperitoneally (i.p.). There were 5 mice in each group. The percentage depression is shown in parenthesis.

Table 7. Molecular weight of anti-oxazolone suppressor factor which inhibits the passive transfer of contact sensitivity

Treatment of passive transfer cells		Contact sensitivity at 24 h	Contact sensitivity at 48 h
No treatment	(positive control)	4·2±0·96	4·2±1·11
No cells transferred	(negative control)	1·5±0·39	1·6±0·48
Incubated in unfractionated factor		2·5±0·75 (63%)	2·3±0·59 (73%)
Amicon fraction > 100,000		4·4±0·28 (<0%)	4·6±1·12 (<0%)
30,000–100,000		3·4±0·70 (30%)	3·2±0·99 (38%)
10,000–30,000		5·1±0·63 (<0%)	5·3±1·08 (<0%)
< 10,000		5·1±0·63 (<0%)	4·9±0·60 (<0%)

Suppressor factor was fractionated by Amicon membrane filtration and concentrated six-fold. 'Passive transfer' cells at $2{\cdot}5 \times 10^7$/ml were incubated in fractions and $4{\cdot}5 \times 10^7$ injected into each recipient.

Table 8. Molecular weight of the α-picryl suppressor factor(s) which inhibits the passive transfer of contact sensitivity and enables peritoneal exudate cells to cause suppression

Cells transferred and their treatment				Contact sensitivity at 24 h		Contact sensitivity at 48 h	
Passive transfer cells		Peritoneal exudate cells					
Eagle's medium	(positive control)		None	3·8±0·86		4·4±0·65	
None	(negative control)		None	1·6±0·48		1·8±0·52	
Amicon fraction	> 100,000		None	3·8±0.20	(0%)	4·8±0·53	(<0%)
	30,000–100,000		None	1·9±0·27	(86%)	2·6±0·27	(69%)
	10,000–30,000		None	3·5±0·48	(14%)	4·7±0·60	(<0%)
	< 10,000		None	3·4±0·63	(18%)	4·5±0·86	(<0%)
No treatment		Amicon faction	> 100,000	2·9±0·60	(40%)	4·1±0·99	(12%)
No treatment			30,000–100,000	1·9±0·66	(86%)	1·9±0·85	(96%)
No treatment			10,000–30,000	3·0±0·68	(36%)	3·8±1·46	(23%)
No treatment			< 10,000	2·6±0·66	(54%)	4·4±0·29	(<0%)

See legend to Table 7. Peritoneal exudate cells were incubated at $1{\cdot}5 \times 10^7$/ml and $1{\cdot}3 \times 10^7$ injected intraperitoneally into recipients which also received untreated passive transfer cells.

serum and complement. Most of the T cells responsible did not adhere to nylon wool. The activity of the nylon-wool adherent cells was abolished by anti-Thyl-2 serum and complement and resided in the T and not in the surface immunoglobulin positive population on separation with anti-immunoglobulin plates. It was due to T cells which had not been eluted from the nylon wool. However, Ts may have shown only slight adherence as some red cells were also found in the adherent fraction. It was concluded that the suppressor cells which depress the efferent stage of the contact sensitivity cells are T cells–Ts-eff (cs).

Production of specific suppressor factor

Mice were injected twice with Ox-TGA or Pic-TGA. Six days later they were painted with the corresponding contact sensitizer and cells harvested on day 7. These cells were incubated for 48 h and their suppressor supernatants studied. Mixed immune lymph node and spleen cells, which transfer contact sensitivity, were incubated in suppressor supernatant for 1 h, washed and then transferred to groups of recipients. Table 4 shows that the α-oxazolone suppressor factor entirely abolished the passive transfer of contact sensitivity to oxazolone but had no effect on passive transfer of contact sensitivity to picryl chloride. The converse was also true. It was concluded that the suppressor factors (SF) were immunologically specific.

Absorption and elution of suppressor factor

Previous experiments with α-picryl suppressor factor produced by the injection of picryl sulphonic acid showed that the factor was specifically absorbed by picryl-albumin linked to sepharose and specifically eluted by the corresponding picryl-ε-aminocaproic acid. Moreover, the factor adhered to, and could be eluted from, concanavalin A linked to sepharose (Asherson *et al.*, 1978). Similar observations were made with α-Ox suppressor factor. Table 5 shows that absorption of the factor once or twice with oxazolonated albumin linked to sepharose reduced its activity, while material eluted with Ox-ε-aminocaproic acid was as active as the original supernatant. This inhibition was not caused by antigen or toxic factors from the Ox-albumin-sepharose as eluates from beads loaded with inactive supernatants had no effect. See line 5 (control eluate) of Table 5. Table 5 also shows that the factor can be absorbed with, and eluted from, Con-A-sepharose.

Arming of peritoneal exudate cells with suppressor factor

Zembala & Asherson (1974) showed that suppressor factor armed peritoneal exudate cells which were then able to suppress the passive transfer of contact sensitivity. Similar observations were made with α-Ox suppressor factor. Table 6 shows that peritoneal exudate cells incubated in suppressor factor and then washed, depressed the passive transfer of contact sensitivity. The degree of inhibition depended on the route of injection of the peritoneal exudate cells. Intraperitoneal injection caused a depression of 87%, while intravenous injection only caused a depression of 55%.

Molecular weight of suppressor factor

The molecular weight of suppressor factor was estimated by Amicon membrane filtration. Table 7 shows that α-Ox suppressor activity appeared in the fraction of notational molecular weight 30,000–100,000. Table 8 shows that suppressor factor produced by the injection of Pic-TGA has a similar molecular weight. It also shows that the factor which arms peritoneal exudate cells and enables them to cause suppression also appears in the same Amicon membrane fraction.

DISCUSSION

Ts-aff, which depress the afferent stage of the reaction to contact sensitizers, are readily produced by painting the skin with contact sensitizer or by injecting dinitrobenzene sulphonic acid (Moorhead, 1976). They are also produced by the injection of haptenized allogeneic cells (Miller, Sy & Claman, 1978b). In contrast, Ts-eff, which depress the efferent stage, are produced by the injection of trinitrobenzene sulphonic acid (picryl sulphonic acid) and the injection of haptenized syngeneic cells (Miller *et al.*, 1978a). Other chemicals with this property have not been described.

The aim of this paper is to develop a general method for producing Ts-eff which depress contact sensitivity and produce suppressor factors. There were two reasons for suspecting that the chemically reactive, water soluble thioethers might produce Ts-eff when injected intravenously. First, the chemically reactive, water soluble compound, picryl sulphonic acid, produces Ts-eff when injected intravenously. This suggested that other chemically reactive, water soluble com-

pounds might have the same property. Second, de Weck, Frey & Geleick (1964) showed in the guinea-pig that the thioether, dinitrophenyl-S-cysteine, caused desensitization while the chemically inactive dinitrophenyl lysine was inactive. This suggested that thioethers might generate suppressor cells, as suppressor cells are often part of the mechanism by which unresponsiveness is effected.

A comment on the chemistry of thioethers may be relevant. Thioethers composed of two alkyl groups (e.g. CH_3-S-CH_3) are unreactive. However, those formed with activating groups, such as the dinitrophenyl group, are chemically reactive. An example is the ability of the thioether in dinitrophenyl-S-cysteine to dinitrophenylate mercaptoethanol (disulphide interchange reaction). It will also dinitrophenylate amino acids and perhaps proteins (Shaltiel, 1967; Frey, de Weck, Geleich & Lergier, 1969). For these reasons the chemically reactive, water soluble thioglycollic acid thioethers of picryl chloride and oxazolone were prepared.

The properties of the suppressor cells produced by Ox-TGA and Pic-TGA were identical to those produced by picryl sulphonic acid. In particular, in both cases, the suppressor cells were T cells which depressed the efferent stage of the contact sensitivity skin reaction–Ts-eff (cs). The suppressor population produced a soluble suppressor factor, which was immunologically specific and had a molecular weight between 30,000 and 100,000 on Amicon membrane filtration. the factor armed peritoneal exudate cells and enabled them to cause suppression. Finally, both α-Ox suppressor factor and α-picryl suppressor factor (produced by the injection of PSA) are specifically absorbed by haptenized albumin linked to sepharose and eluted with haptenized-EACA. Moreover, they adhered to and were eluted from Con A-sepharose. The implication is that picryl sulphonic acid, Ox-TGA and Pic-TGA all produce the same type of Ts-eff. This may be related to their chemical reactivity and the administration of high doses.

T suppressor factor affects passive transfer in two distinct ways. It binds to an adherent theta negative peritoneal exudate cell (Ptak, Zembala, Asherson & Marcinkiewicz, 1980). This presumptive macrophage then releases a nonspecific inhibitor of about 10,000 daltons when exposed to antigen. This nonspecific factor, unlike T suppressor factor, does not bind to immune cells at 4°, but blocks the passive transfer of contact sensitivity on incubation at 37° (Ptak, Zembala, Hanczakowska-Recklicka & Asherson, 1978).

The simple view that the T suppressor factor acts directly on the actual cells which transfer contact sensitivity is probably wrong. The evidence against this view is that immune cells are not affected by T suppressor factor after adult thymectomy or treatment with cyclophosphamide, and that this resistance is reversed by normal immune cells. This suggests that T suppressor factor acts indirectly through an auxiliary cell(s) which is sensitive to cyclophosphamide and adult thymectomy. This cell is probably the same as the auxiliary cell of Sy *et al.* (1979).

A priori, T suppressor factor and the non-specific factors might act by inhibiting the movement of passive transfer cells to the test site, or by an anti-inflammatory effect or by the inhibition of lymphokine production. There is direct evidence for the last possibility. Immune cells liberate a lymphokine following exposure to antigen which inhibits the migration of T cells *in vitro*. This lymphokine is not produced following exposure to T suppressor factor (Zembala, Kowalczyk, Asherson & Noworolski, 1980).

The anatomical site for the production of Ts-eff (cs) remains to be defined. It is unlikely that access of antigen to the spleen or thymus is required for their production. For instance, Ts-eff (cs) can be found in mice injected with picryl sulphonic acid a few days, although not a few weeks, after adult thymectomy. Splenectomy also has no effect (Asherson, Zembala, Mayhew & Goldstein, 1976).

It is possible that association of haptene with a particular major histocompatibility complex (MHC) antigen is required for the generation of Ts-eff, while a different association is required for the generation of Ts-aff. The experiments of Miller *et al.* (1978a, b) can be viewed in this context. They showed that dinitrophenylated syngeneic, but not allogeneic, lymphoid cells generated Ts-eff (cs).

The present system has features reminiscent of some drug reactions in humans. It is a paradox that patients injected intravenously with grams of chemically reactive drugs such as penicillin and ampicillin usually fail to develop drug reactions. This is despite the fact that penicillin regularly binds to red blood cells and presumably to other cells *in vivo* (Levine & Redmond, 1967). A possible explanation is that the injection of high doses of these agents generates Ts-eff (cs) or other suppressor cells.

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