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THE MECHANISM OF IMMUNOLOGICAL UNRESPONSIVENESS TO PICRYL CHLORIDE AND THE POSSIBLE ROLE OF ANTIBODY MEDIATED DEPRESSION

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SUMMARY

Mice were rendered specifically unresponsive to picryl chloride by pretreatment with picryl sulphonic acid. These mice fail to develop contact sensitivity, as judged by increment of ear thickness, when subsequently sensitized on the abdomen and challenged 6 days later with picryl chloride on the ear.

These mice were not in a pure state of classical immune tolerance. This was shown in two ways.

(1) Cells from unresponsive donors were injected intravenously into normal CBA mice. The mice were then sensitized on the same day and challenged 6 days later. 'Unresponsive' (but not a variety of control) lymph node cells impaired the development of contact sensitivity to picryl chloride. The impairment was immunologically specific and 'unresponsive' cells did not impair the development of contact sensitivity to 'oxazolone'.

(2) Unresponsive mice were irradiated and restored with 'unresponsive' bone marrow cells. They regained immune competence to picryl chloride when injected with normal lymph node cells and sensitized on the same day, and failed to regain competence when injected with unresponsive lymph node cells. The distinctive finding was that the injection of a mixture of normal and unresponsive lymph node cells failed to restore immune competence. Similar results were obtained when irradiated but otherwise normal recipients were used. Unresponsive cells also impaired the passive (adoptive) transfer of contact sensitivity.

These results show that lymph node cells from mice which are unresponsive to picryl chloride actively and specifically impair the induction or manifestation of contact sensitivity to picryl chloride. It was concluded that this form of unresponsiveness is not classical tolerance and the hypothesis is put forward that the unresponsiveness is due at least in part to antibody mediated depression of contact sensitivity.

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INTRODUCTION

Mice repeatedly injected with picryl sulphonic acid become unresponsive and fail to develop contact sensitivity when subsequently sensitized, and challenged with picryl chloride (Asherson & Ptak, 1970). Three mechanisms may explain this specific immunological unresponsiveness: (a) immunological tolerance in which the antigen is thought to inhibit or delete a clone of cells directly; (b) immunological enhancement (i.e. antibody mediated depression of delayed hypersensitivity) in which antibody limits the development or manifestation of delayed hypersensitivity; (c) immunological unresponsiveness mediated by antibody and antigen (Feldmann & Diener, 1970).

Immune tolerance is defined as a passive state in which a clone of cells is deleted or inactive; while immune enhancement is defined as an active state in which the antibody produced by lymphoid cells depresses the immune responses (or manifestation of the immune responses) of other lymphoid cells.

Two types of cell transfer studies can be used to distinguish between immune tolerance and immune enhancement. Cells transferred from tolerant to normal animals would not be expected to impair immune responses. In contrast, cells from animals showing immune enhancement would be expected to impair the induction or manifestation of immune responses because of the production of blocking antibody. The first part of this paper shows that the injection of lymph node cells from mice rendered unresponsive to picryl chloride impairs contact sensitivity in normal mice following skin painting with picryl chloride. A similar approach was used by Crowle & Hu (1969).

Comparable studies can be made by transferring 'normal' and 'unresponsive' lymph node cells to mice whose immune system has been destroyed by irradiation.

This paper shows that mice which have been rendered unresponsive to picryl chloride and then irradiated will respond to picryl chloride following transfer of normal lymph node cells. Unresponsive lymph node cells fail to restore competence. However, the distinctive finding is that unresponsive lymph node cells interfere with the behaviour of normal lymph node cells. The most likely explanation is that unresponsiveness to picryl chloride (induced by picryl sulphonic acid) in the mouse is not an example of pure, classical tolerance, and is due, at least in part, to antibody mediated depression of delayed hypersensitivity.

MATERIALS AND METHODS

The general methods are given in Asherson & Zembala (1970).

Animals

CBA mice of both sexes, bred locally and bought from Animal Laboratory Supplies, were used. Only one sex was used in any one experiment. Mice were labelled by clipping the fur and dyeing with carbol-fushsin and not by ear punch, and were assigned to groups with the help of a table of random numbers.

Induction of unresponsiveness

Mice were injected five times with 0·5 ml 1% solution of trinitrobenzene sulphonic acid (picryl sulphonic acid, British Drug Houses, Poole, England) dissolved in saline and neutralized with sodium bicarbonate, twice weekly. The mice were then left for 4–8 weeks.

In some experiments a few mice were sensitized to show that unresponsiveness was complete and were not used further.

Donor mice

Lymph node cells were prepared from the inguinal and shoulder girdle lymph nodes of normal and unresponsive mice. Cervical and mesenteric lymph nodes were also used in experiments 2 and 3. In some experiments cells from mice sensitized 7 days previously with picryl chloride or 3% 2-ethoxymethylene-4-phenyloxazolone (oxazolone), obtained from British Drug Houses, were used. Bone marrow cells were prepared from unresponsive donors unless otherwise stated.

Cell transfer

In the first series of experiments (experiments 2–7) the recipients were normal CBA mice given varying numbers of lymph node cells from normal mice or mice rendered unresponsive with picryl sulphonic acid. In the later experiments the recipients were unresponsive mice (or in Table 7, normal mice) irradiated on the day of transfer with 1000 r. All these recipients except for certain negative controls received 'unresponsive' bone marrow cells. They also received varying numbers of normal and unresponsive lymph node cells intravenously. The cells were mixed together before injection.

Sensitization

In experiments 1–7 picryl chloride (Hopkin and Williams) was purified by washing with sodium bicarbonate followed by water and then recrystallized from ethanol. 5% in alcohol was used for sensitization. In other experiments 7% unrecrystallized picryl chloride was used. The mice were sensitized within 2 hr of the cell transfer with 0·1 ml picryl chloride in alcohol on the skin of the clipped abdomen. Sensitized and unsensitized mice were kept in separate cages.

Challenge

The mice were challenged on day 6 and at later times with 1% picryl chloride or 2% oxazolone in olive oil on both sides of both ears. Some mice were resensitized with 5 or 7% picryl chloride and later rechallenged.

Quantitation

The thickness of the ears was measured before and 24 hr after challenge, with an engineer's micrometer and the results expressed as the increment of ear thickness in units of 10^{-3} cm $\pm$ the standard deviation.

RESULTS

Specificity of immunological unresponsiveness to picryl chloride (*Table* 1)

Mice were rendered unresponsive to picryl chloride while control mice were injected with saline. The mice were then sensitized with picryl chloride or oxazolone and challenged 6 days later with the corresponding antigen. Table 1 shows that normal mice gave a mean increment of ear thickness at 24 hr of 8·4 units, while unresponsive mice showed a mean increment of $-0{\cdot}2$ units. In contrast, normal and unresponsive mice sensitized to oxazolone showed mean

reactions of 13·9 and 11·1 units. It was concluded that unresponsiveness to picryl chloride induced by the injection of picryl sulphonic acid was immunologically specific.

Passive transfer of unresponsive cells into normal recipients (*Table* 2)

The effect of unresponsive cells on the immunological behaviour of normal mice was investigated in a series of experiments. In the first experiment unresponsive cells were injected into some mice while other (control mice) were left uninjected. The mice were then sensitized to picryl chloride and challenged 6 days later.

Experiment 2, Table 2, shows that the control mice gave a mean increment of ear thickness of 9·9 units while mice which had received $2–5 \times 10^7$ unresponsive lymph node cells gave a mean increment of only 6·0. This difference was statistically significant by the double tail Mann-Whitney U test.

TABLE 1. Specificity of immunological unresponsiveness to picryl chloride: skin reactions to picryl chloride and oxazolone in normal mice and mice unresponsive to picryl chloride

State of mice	Antigen used to sensitize and challenge	Increment of ear thickness at 24 hr in units of 10^{-3} cm	
		Sensitized	Control*
Normal	Picryl chloride	8·4±2·15	−0·4±1·2
Unresponsive to PCl†	Picryl chloride	−0·2±0·47	−0·8±0·91
Normal	Oxazolone	13·9±2·97	3·5±1·50
Unresponsive to PCl†	Oxazolone	11·1±1·34	3·0±0·77

The figures show the mean increment of ear thickness ± standard deviation. Each figure is based on five mice.

* Non-specific swelling in mice which were not sensitized.

† These mice were pretreated with picryl sulphonic acid.

Experiment 3 confirms that 3 and 5×10^7 unresponsive cells reduced contact sensitivity. Cells from donors immunized to picryl chloride had no effect. It was concluded that lymph node cells from unresponsive mice depressed the development of contact sensitivity in normal mice. The next section shows that this effect was immunologically specific, i.e. cells from donors rendered unresponsive to picryl chloride depressed contact sensitivity to picryl chloride but had little or no effect on contact sensitivity to oxazolone.

Control experiments (*Table* 3)

Experiment 4, Table 3, confirms that 'unresponsive' cells depress contact sensitivity to picryl chloride and shows that normal cells have no effect. The mean increment of ear thickness in mice sensitized to picryl chloride was 12·6 units. This was reduced to 9·1 units by the injection of 'unresponsive' cells. However, normal cells caused virtually no reduction (mean increment 12·1 units).

Experiment 5, Table 3, shows that normal cells and cells sensitized to oxazolone failed to depress contact sensitivity to picryl chloride. Experiment 6 shows that unresponsive cells

TABLE 2. Effect of passive transfer of unresponsive and other lymph node cells on the ability of normal mice to show contact sensitivity to picryl chloride following sensitization

Experiment	Lymph node cells transferred	Increment of ear thickness at 24 hr	
		Sensitized	Control
2	Nil (+ve control)	9·9±2·63 (10)	1·7±0·65 (10)
	5×10^7 unresponsive to PCl	5·8±0·93 (4)	
	2×10^7 unresponsive to PCl	6·4±0·20 (3)	1·4±0·32 (4)
3	Nil (+ve control)	10·4±3·10 (5)	1·5±0·76 (4)
	3×10^7 unresponsive to PCl	5·6±1·0 (5)	
	5×10^7 unresponsive to PCl	5·5±0·48 (4)	1·6±0·26 (4)
	3×10^7 sensitized to PCl	9·7±2·95 (5)	

Normal mice were injected with the stated number of lymph node cells. Some mice were then sensitized and their reactions on challenge 6 days later are shown in the column 'sensitized'. Other mice were left unsensitized as non-specific controls and their reactions on challenge are shown in the column 'control'. The figures are the mean and standard deviation of the increment in ear thickness in units of 10^{-3} cm. The number of mice is shown in parentheses.

TABLE 3. Effect of passive transfer of unresponsive and other lymph node cells on the ability of normal mice to show contact sensitivity to picryl chloride and oxazolone following sensitization (control experiments)

Experiment	Lymph node cells transferred	Donors	Increment of ear thickness at 24 hr	
			Sensitized	Control
4	Nil (+ve control)	Sensitized with PCl	12·6±2·82 (5)	1·8±0·75 (5)
	3×10^7 unresponsive to PCl	Sensitized with PCl	9·1±0·27 (4)	1·6±0·24 (4)
	3×10^7 normal	Sensitized with PCl	12·1±1·21 (4)	1·8±0·54 (4)
5	Nil (+ve control)	Sensitized with PCl	6·9±1·99 (5)	0·1±0·36 (5)
	5×10^7 normal	Sensitized with PCl	6·7±1·22 (3)	1·0±0·70 (5)
	3×10^7 normal	Sensitized with PCl	6·9±1·39 (6)	0·4±0·24 (5)
	3×10^7 sensitized to Ox	Sensitized with PCl	6·9±2·36 (5)	1·4±0·62 (5)
6	Nil (+ve control)	Sensitized with PCl	13·7±1·51 (5)	1·5±1·02 (5)
	3×10^7 unresponsive to PCl	Sensitized with PCl	8·4±1·11 (4)	1·7±0·30 (4)
	Nil (+ve control)	Sensitized with Ox	17·9±1·44 (5)	4·3±1·47 (5)
	3×10^7 unresponsive to PCl	Sensitized with Ox	20·5±1·73 (4)	4·5±1·40 (4)
7	Nil (+ve control)	Sensitized with Ox	17·1±2·03 (6)	6·3±1·49 (5)
	3×10^7 unresponsive to PCl	Sensitized with Ox	15·9±1·34 (4)	5·9±1·74 (4)
	3×10^7 normal	Sensitized with Ox	19·6±1·35 (4)	5·7±3·07 (3)

Normal mice were injected with the stated number of normal lymph node cells or cells unresponsive to picryl chloride. Some mice were then sensitized and challenged with picryl chloride while others were sensitized and challenged with the control antigen–oxazolone. The reactions on challenge 6 days later are shown in the column 'sensitized'. Other mice were left unsensitized as non-specific controls and their reactions on challenge are shown in the column 'control'. The figures are the mean and standard deviation of the increment in ear thickness in units of 10^{-3} cm. The number of mice is shown in parentheses.

did not depress contact sensitivity to oxazolone. This is confirmed in experiment 7 in which there is a suggestion that normal cells increase the response to oxazolone.

It was concluded that lymph node cells unresponsive to picryl chloride cause specific depression of contact sensitivity to picryl chloride when injected into normal mice.

Ability of unresponsive cells to prevent the restoration of immune competence by normal cells (*Table* 4)

Normal lymph node cells restore immune competence to picryl chloride in unresponsive mice, providing the recipient mice are irradiated before cell transfer (Asherson & Ptak, 1970). The following experiments investigated the restoration of immune competence to picryl chloride in unresponsive, irradiated recipients. Throughout the text the term *immune competence* refers to the ability to show contact sensitivity to picryl chloride following sensitization and challenge.

The first experiment confirmed that normal lymph node cells restore immune competence to unresponsive, irradiated (1000 r) recipients (protected with 2×10^6 'unresponsive' bone marrow cells) and showed that 'unresponsive' lymph node cells failed to restore immune competence. It also investigated the effect of 'unresponsive' cells on the restoration of immune competence by normal lymph node cells.

Table 4 shows that mice given 5×10^6 normal lymph node cells gave a mean increment of ear thickness of 5·8 units. In contrast, mice given the same number of normal cells together with 10×10^6 'unresponsive' cells showed a mean increment of ear thickness of only 1·8 units. 5×10^5 and 5×10^4 normal lymph node cells also caused partial restoration of immune competence and this did not occur when the normal cells were given together with 'unresponsive' lymph node cells. It was concluded that 10×10^6 unresponsive lymph node cells virtually abolished the restoration of immune competence by normal lymph node cells.

Effect of varying the number of 'unresponsive' cells on the restoration of immune competence by normal lymph node cells (*Table* 5)

Unresponsive, irradiated recipients were given a mixture of 'unresponsive' bone marrow cells and a constant number of normal lymph node cells (5×10^5). The mixture also contained a varying number of 'unresponsive' lymph node cells. Table 5 shows that 10×10^6 'unresponsive' lymph node cells abolished restoration of immune competence while even 1×10^4 'unresponsive' cells slightly depressed restoration. It was concluded that the restoration of immune competence by normal lymph node cells was impaired by 1/5 to 1/50 of their number of unresponsive cells.

Some of the mice in this experiment died, probably as a result of the irradiation. This prevented a firm conclusion being drawn when the mice were resensitized and rechallenged 14 days after transfer. It appeared, however, that at that time the 'unresponsive' cells caused only a slight depression in the restoration of immune competence.

The effect of delayed sensitization (*Table* 6)

It seemed possible that 'unresponsive' cells might interfere, in these irradiated recipients, with normal cells by competing for an anatomical site or metabolite needed for cell division. The following experiment excluded this possibility. Cells were injected into unresponsive, irradiated recipients and then allowed to divide for 14 days before sensitization with picryl chloride. Table 6 shows that 5×10^5 lymph node cells restored contact sensitivity to picryl

chloride (mean increment of ear thickness 3·5 units) while 10×10^6 unresponsive cells given together with the normal cells depressed this restoration (mean increment 1·3 units). As in the last experiment, when the mice were resensitized, and rechallenged at 30 days, the 'unresponsive' cells caused only a slight depression in the restoration of immune competence.

TABLE 4. Effect of unresponsive lymph node cells on the restoration of contact sensitivity to unresponsive, irradiated mice by normal lymph node cells

Number and type of lymph node cells transferred			Mean increment of ear thickness at 24 hr
Normal	Unresponsive		
5×10^6	0	Sensitized	5·8 ±0·78 (3)
5×10^6	1×10^7	Sensitized	1·8 ±1·30 (4)
5×10^5	0	Sensitized	3·3 ±0·99 (4)
5×10^5	1×10^7	Sensitized	0·45±0·69 (4)
5×10^4	0	Sensitized	3·1 ±1·03 (4)
5×10^4	1×10^7	Sensitized	1·1 ±0·74 (3)
5×10^3	0	Sensitized	2·2 ±1·27 (2)
5×10^3	1×10^7	Sensitized	1·6 ±0·79 (4)
0	0	Sensitized	0·26±0·56 (4)
0	1×10^7	Sensitized	0·3 ±0·11 (2)
−ve control in unsensitized mice			
5×10^6	0	Unsensitized	1·5 ±0·76 (4)
0	1×10^7	Unsensitized	0·1 ±0·02 (4)
−ve control for completeness of unresponsiveness*			
0	0	Sensitized	0·01±0·18 (4)
0	0	Unsensitized	0·01±0·086 (4)
+ve control for reactivity to picryl chloride†			
0	0	Sensitized	7·1 ±2·07 (4)
0	0	Unsensitized	2·4 ±1·01 (4)

Mice which had been rendered unresponsive to picryl chloride, were irradiated and injected with 2×10^6 'unresponsive' bone marrow cells and the stated number of normal and 'unresponsive' lymph node cells. They were sensitized to picryl chloride and challenged 6 days later. The figures show the mean increment in ear thickness and the standard deviation in units of 10^{-3} cm. The number of mice is shown in parentheses.

* Unirradiated mice.
† Normal mice.

Depression of restoration of immune competence in irradiated, but otherwise normal recipients (Table 7)

It is convenient to use irradiated recipients which have not been rendered unresponsive to picryl chloride. Table 7 shows that irradiated mice restored with 5×10^5 normal lymph node cells gave a mean reaction to picryl chloride of 5·1 units. $1–5 \times 10^5$ 'unresponsive' lymph node cells depressed and 5×10^6 'unresponsive' cells abolished this restoration. It was concluded that 'unresponsive' cells depressed the restoration of immune competence by normal lymph node cells both in irradiated, but otherwise normal, recipients and in recipients which had been rendered unresponsive to picryl chloride before irradiation.

TABLE 5. Effect of number of 'unresponsive' lymph node cells on the restoration of contact sensitivity to unresponsive, irradiated mice by normal lymph node cells

Number and type of lymph node cells transferred			Mean increment of ear thickness at 24 hr on challenge at	
Normal	Unresponsive		6 days	14 days
5×10^5	0	Sensitized	4·95 ± 1·69 (4)	7·1 ± 1·52 (2)
5×10^5	1×10^7	Sensitized	0·5 ± 0·25 (3)	4·1 ± 0·39 (2)
5×10^5	1×10^6	Sensitized	0·85 ± 1·78 (4)	5·2 ± 0·75 (4)
5×10^5	1×10^5	Sensitized	2·05 (1)	5·5 (1)
5×10^5	1×10^4	Sensitized	2·9 ± 0·37 (3)	6·3 ± 1·60 (3)
0	1×10^7	Sensitized	0·65 ± 0·23 (4)	2·7 ± 0·32 (2)
− ve control for non-specific swelling				
5×10^5	0	Unsensitized	0·15 ± 0·11 (4)	1·6 ± 0·91 (3)
+ ve control for reactivity to picryl chloride*				
0	0	Sensitized	8·3 ± 1·36 (3)	18·2 ± 2·65 (3)
0	0	Unsensitized	1·5 ± 0·50 (4)	6·7 ± 1·21 (4)†

See legend to Table 3. All the irradiated mice received 3×10^6 unresponsive bone marrow cells and the stated number of normal and unresponsive lymph node cells. They were sensitized on the same day and resensitized on day 8. They were challenged on days 6 and 14. The number of mice is shown in parentheses.

* Normal mice.

† The large increment in ear thickness is probably due to sensitization as a result of the first challenge.

TABLE 6. Effect of delaying the time of sensitization on the ability of unresponsive cells to prevent the restoration of immune competence by normal lymph node cells

Number and type of lymph node cells injected			Mean increment of ear thickness at 24 hr on challenge at:	
Normal	Unresponsive		20 days	30 days
5×10^5	0	Sensitized	3·5 ± 0·93 (3)	3·7 ± 1·26 (3)
5×10^5	1×10^7	Sensitized	1·3 ± 0·25 (3)	2·1 ± 0·70 (3)
0	1×10^7	Sensitized	0·8 ± 0·3 (3)	0·9 ± 0·86 (3)
− ve control for non-specific swelling				
5×10^5	0	Unsensitized	0·6 ± 0·28 (4)	0·54 ± 0·49 (4)

All the irradiated mice received 2×10^6 unresponsive bone marrow cells and the stated number of normal and unresponsive lymph node cells. They were sensitized on days 14 and 23 and challenged on days 20 and 30.

The effect of unresponsive cells on the passive (adoptive) transfer of contact sensitivity by sensitized lymph node cells (Table 8)

The previous experiments describe the depressing effect of 'unresponsive' cells on the restoration of immune competence by normal lymph node cells. The following experiment shows that unresponsive cells also depress the passive (adoptive) transfer of contact sensitivity

TABLE 7. Effect of unresponsive lymph node cells on the restoration of contact sensitivity to irradiated, but otherwise normal mice, by normal lymph node cells

Number and type of lymph node cells transferred			Mean increment of ear thickness at 24 hr
Normal	Unresponsive		
5×10^3	0	Sensitized	1·4 ±0·60 (2)
5×10^4	0	Sensitized	2·2 ±0·89 (4)
5×10^5	0	Sensitized	5·1 ±2·67 (6)
5×10^5	1×10^5	Sensitized	2·9 ±0·70 (2)
5×10^5	5×10^5	Sensitized	2·4 ±0·53 (2)
5×10^5	5×10^6	Sensitized	0·9 ±1·25 (3)
0	5×10^6	Sensitized	1·0 ±0·27 (5)
0	0	Sensitized	0·9 ±0·51 (5)
−ve control for non-specific swelling			
5×10^5	5×10^6	Unsensitized	0·17±0 77 (3)
0	0	Unsensitized	0·24±0·78 (4)
0	0	Sensitized	1·4 ±0·60*(5)

In this experiment irradiated, but otherwise normal recipients were used instead of recipients which had been rendered unresponsive to picryl chloride before irradiation.

* These mice received 2×10^6 normal bone marrow cells instead of unresponsive bone marrow cells.

TABLE 8. Effect of unresponsive lymph node cells on the passive (adoptive) transfer of contact sensitivity by lymph node cells from donors sensitized to picryl chloride

Number and type of lymph node cells transferred			Mean increment of ear thickness at 24 hr	
Immune	Unresponsive		6 days	13 days
$1{\cdot}5 \times 10^8$	0	Unsensitized	5·2±0·11 (2)	2·2±0·46 (2)
3×10^7	0	Unsensitized	3·4±0·88 (6)	3·1±1·30 (6)
3×10^7	$2{\cdot}5 \times 10^7$	Unsensitized	1·0±0·72 (6)	0·4±1·01 (6)
0	$2{\cdot}5 \times 10^7$	Unsensitized	0·3±0·39 (5)	−0·3±0·43 (5)
−ve control for non-specific swelling				
0	0	unsensitized	0·7±0·45*(5)	−0·5±1·51*(5)

As in the last experiment irradiated, but otherwise normal, mice were used as recipients. They received 2×10^6 unresponsive bone marrow cells and varying numbers of lymph node cells from donors sensitized to picryl chloride 7 days previously and donors unresponsive to picryl chloride. They were challenged 6 and 13 days after transfer. In contrast to the other experiments the recipient mice were not sensitized with picryl chloride after transfer.

* These mice received normal lymph node cells.

by sensitized lymph node cells. Donors sensitized with picryl chloride 7 days previously were used for the passive (adoptive) transfer of contact sensitivity. A mixture of these sensitized lymph node cells and unresponsive bone marrow cells were injected into irradiated

recipients. Some of the recipients also received unresponsive lymph node cells. The mice were challenged 6 days after transfer. In contrast to the previous experiment they were not sensitized to picryl chloride.

Table 8 shows that the mean reaction in mice given cells from sensitized donors was 3·4 units. This was depressed to 1·0 units when unresponsive lymph node cells were also given. It was concluded that unresponsive cells depressed the adoptive transfer of contact sensitivity. This depression was still apparent on rechallenge at 13 days.

DISCUSSION

The results show that mice can be rendered specifically unresponsive to picryl chloride by the injection of picryl sulphonic acid. This depression does not behave like classical immunological tolerance as lymph node cells from unresponsive mice depress the ability of normal mice to develop contact sensitivity following sensitization with picryl chloride. This effect was immunologically specific as normal lymph node cells and lymph node cells from animals immunized with oxazolone had no depressant effect.

These results were confirmed in a transfer system. It was first established that normal lymph node cells restored immune competence to picryl chloride in unresponsive, irradiated mice, while lymph node cells from mice which had been rendered unresponsive to picryl chloride did not restore immune competence to this antigen. The interesting finding was that 'unresponsive cells' depressed the restoration of immune competence by normal cells. This implied that the 'unresponsive' cells blocked either the induction or the manifestation of contact sensitivity to picryl chloride. The mechanism may be: (a) Competition between 'unresponsive' and normal lymph node cells for critical sites or metabolites. This is unlikely as the restoration of immune competence by normal lymph node cells is impaired by 1/5 to 1/50 of their number of unresponsive cells. (b) Carry-over of tolerogenic antigen by the unresponsive cells. This is unlikely as 'unresponsive' cells depress the restoration of immune competence in irradiated, *unresponsive* recipients which have already been exposed to large quantities of antigen. (c) Non-specific depression of inflammatory responses. This is unlikely as normal cells and cells immunized to oxazolone fail to depress contact sensitivity to picryl chloride. (d) Antibody mediated depression of the induction or manifestation of contact sensitivity. This hypothesis offers a plausible mechanism whereby a few cells may alter the behaviour of a large number of other cells. It suggests that cells which are unresponsive as judged by failure to produce contact sensitivity liberate antibody (either spontaneously or on exposure to antigen) which blocks the induction or manifestation of contact sensitivity.

There are several systems in which unresponsiveness caused by antigen is due, at least in part, to antibody mediated depression. This is seen in unresponsiveness to sheep red cells in the rat (Rowley & Fitch, 1964) and bovine serum albumin in the rabbit (Frei, 1969). See Uhr & Möller (1968). Antibody mediated depression of the induction or manifestation of cell mediated immunity has been demonstrated for unresponsiveness to skin and tumour transplants (Voisin, Kinsky & Maillard, 1968) and by cell transfer in mice with selective depression of delayed hypersensitivity (Crowle & Hu, 1969).

Recent work of Hellström, Hellström & Sjogren (1970), Hellström *et al.* (1970) and Hellström, Allison & Hellström (1971) has emphasized the role of factors (antibodies) which block the *in vitro* manifestation of cell mediated immunity in animals with apparent tolerance

to tumours and skin grafts. The question arises whether other examples of unresponsiveness which are usually regarded as pure immunological tolerance are actually due to antibody mediated depression of the induction or manifestation of immune responses.

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