Immunology, 1973, **24**, 885.

Contact Sensitivity in the Mouse

IX. THE ROLE OF IMMUNOLOGICAL AND NON-IMMUNOLOGICAL INFLAMMATION IN THE MOVEMENT OF LYMPHOCYTES TO IMMUNIZED LYMPH NODES

G. L. Asherson and R. M. R. Barnes

Division of Immunology, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

(*Received* 23*rd September* 1972; *accepted for publication* 30*th October* 1972)

Summary. Normal mice were immunized with picryl chloride. ^{51}Cr-labelled normal lymph node cells were injected 1–10 days afterwards and the net arrival of radioactivity at the draining lymph nodes estimated. The net arrival at normal unimmunized lymph nodes was 5·3 per cent of the injected dose. This rose to 13·4 per cent 1 day after immunization and then gradually declined.

This increased arrival caused by picryl chloride was reduced in mice rendered unresponsive by the repeated injection of picryl sulphonic acid or given a single injection of picryl sulphonic acid 1–6 days before immunization. Blast cells labelled with ^{125}IUDR from lymph nodes immunized with an unrelated agent also showed an increased arrival at draining lymph nodes. This increased arrival was reduced in unresponsive mice painted with picryl chloride.

A single intravenous injection of picryl sulphonic acid increased the arrival of normal lymph node cells at lymph nodes 1 day later and this effect was also diminished in mice rendered unresponsive by repeated injections of picryl sulphonic acid.

The arrival of normal lymph node cells at the spleen was reduced by painting with picryl chloride or by a single injection of picryl sulphonic acid given a few days beforehand.

Painting the ears of unimmunized mice with picryl chloride increased the arrival of normal lymph node cells and blast cells at the ears. However, the arrival was unaffected by pretreatment with picryl sulphonic acid.

It was concluded that the increased arrival of cells in lymph nodes caused by picryl chloride and picryl sulphonic acid had both an immunological and a chemical inflammatory component. In contrast the arrival at painted ears lacked an immunological component. This provided evidence for antibody, or antigen-sensitive cells in apparently unimmunized mice which rapidly release pharmacologically active agents on exposure to antigen.

INTRODUCTION

Zatz and Lance (1971a) showed that a range of immunogens increased the arrival of lymphocytes at the draining lymph nodes while non-immunogenic materials lacked this ability. Zatz and Lance (1971b) found that the ability of skin grafts to increase the arrival

of lymphocytes at the draining lymph nodes shortly before the time of graft rejection was diminished by the neonatal induction of tolerance. Contact sensitizing agents also increase the arrival of lymphocytes at immunized lymph nodes (Asherson and Allwood, 1972). This increased arrival occurs within 18 hours of immunization and diminishes by 72 hours. The increased arrival of lymphocytes may be due either to the irritant effects of the contact sensitizing agent related to its chemical reactivity and ability to combine with cell surfaces or to its immunogenicity. These two modes of action can be distinguished by studying the ability of lymphocytes to arrive at lymph nodes immunized by contact sensitizing agents in normal mice and in mice rendered specifically unresponsive to picryl chloride. This paper shows that at least a part of the arrival of lymphocytes at draining lymph nodes within 44 hours of a primary immunization with picryl chloride is due to an immunological phenomen and not only to chemical inflammation.

MATERIALS AND METHODS

Animals

Male CBA mice, 2–6 months old, were obtained from the Animal Division, C.R.C., or from Animal Services Laboratory Ltd.

Immunization

Contact sensitivity: 0·1 ml of either 3·0 per cent 4-ethoxymethylene-2-phenyl-oxazolone ('oxazolone' or OX) (British Drug Houses) or 5·0 per cent recrystallized picryl chloride (PIC-Cl) (Hopkins and Williams) in absolute ethanol was applied to the clipped abdomen.

Unresponsiveness to picryl chloride

Picryl sulphonic acid (PSA) (Sigma), neutralized with sodium bicarbonate, was injected intravenously either in a single dose of 5 mg, or 5 mg given repeatedly up to five times over 2–3 weeks (Asherson, Zembala and Barnes, 1971).

Ear challenge

A total of four drops (0·08 ml) of 1·0 per cent PIC-Cl or OX dissolved in olive oil by warming were delivered on to both sides of both ears from a 25-gauge needle and syringe held vertically and spread with a plastic rod.

PREPARATION OF LABELLED CELL SUSPENSIONS

Normal lymph node cells

The inguinal, subscapular, brachial and cervical lymph nodes were removed from unimmunized mice and pooled. Cell suspensions were prepared in medium 199 containing penicillin and streptomycin with sodium bicarbonate and 10 per cent foetal calf serum (Flow Laboratories) by gently pressing through a metal sieve with a metal plunger and then filtering through bolting cloth (John Stannier, Manchester; normal quality 17/80) to remove debris. Viability was assessed by eosin exclusion and usually exceeded 75–80 per cent.

Normal lymph node cell (LNC) suspension were labelled with $Na_2{}^{51}CrO_4$ (specific activity 2·0–5·0 mCi/μg, ^{51}Cr, Radiochemical Centre, Amersham) and prepared for injection following Asherson and Allwood (1972). Experiments showed that the percentage

arrival of LNC at lymph nodes 24 hours after immunization was constant over the range of injected cells 1×10^5 to 1×10^7. For convenience, however, a standard intravenous injection of 2×10^6 live cells in 0·5 ml was used.

Immunized lymph node cells

The inguinal and brachial lymph nodes from mice sensitized with OX 3 days previously were removed, pooled and a suspension made.

Three-day OX LNC were labelled with ^{125}IUDR (specific activity 1–6 mCi/μg, Radiochemical Centre, Amersham). 10·0 μCi in saline were added to 10^8 cells in 5·0 ml of medium, incubated at 37° for 30 minutes, washed three times and filtered. 2×10^6 live cells in 0·5 ml were injected intravenously.

Removal of tissues and radioactivity counting

Eighteen hours after cell injection, recipients were killed by cervical dislocation and the following tissues removed: (1) lymph nodes (inguinal and brachial but not the subscapular); (2) spleen; (3) ears (both ears were cut off at the ear base where the cartilage thickens). The tissues and aliquots of injected cells were counted in a Packard Autogamma Spectrometer model 3002.

Statistics

The results are expressed as the mean and standard deviation (n–1 degrees of freedom) of the tissue ^{51}Cr expressed as a per cent of the injected dose. Levels of significance were determined by Student's t-test.

RESULTS

ARRIVAL OF LYMPH NODE CELLS AT IMMUNIZED LYMPH NODES AT VARIOUS TIMES AFTER CELL INJECTION

One group of mice was immunized with PIC-Cl 24 hours before the injection of ^{51}Cr-labelled normal lymph node cells while control mice were left unimmunized. The mice were killed 2–48 hours after cell injection and the radioactivity of the draining

TABLE 1

INCREASED ARRIVAL OF NORMAL LYMPH NODE CELLS AND BLAST CELLS AT LYMPH NODES AFTER A SINGLE INJECTION OF PICRYL SULPHONIC ACID: THE EFFECT OF UNRESPONSIVENESS

Treatment of recipients	Percentage arrival of ^{51}Cr (cells) at draining lymph nodes		Percentage arrival of ^{125}I (cells) at draining lymph nodes	
	Normal	Unresponsive	Normal	Unresponsive
Experiment 1				
Nil (untreated control)	6·9 ± 0·63	6·4 ± 0·86	—	—
PSA at −24 hours	*9·1 ± 1·67	7·1 ± 1·11	—	—
PSA at −72 hours	6·2 ± 0·9	6·4 ± 1·28	—	—
Experiment 2				
Nil (untreated control)	3·6 ± 0·68	2·8 ± 0·67	0·25 ± 0·06	0·19 ± 0·05
PSA at −6 hours	*5·0 ± 1·0	3·0 ± 1·08	1·03 ± 0·11	0·17 ± 0·03

* $P < 0·05$. † $P < 0·001$ statistically significantly different from nil (untreated control) group.

The figures show the mean net arrival of ^{51}Cr-labelled normal lymph node cells, or ^{125}IUDR-labelled lymph node cells taken 3 days after immunization with oxazolone, expressed as a percentage of the radioactivity injected, ± S.D. Each figure is based on four (experiment 1) or five (experiment 2) recipients.

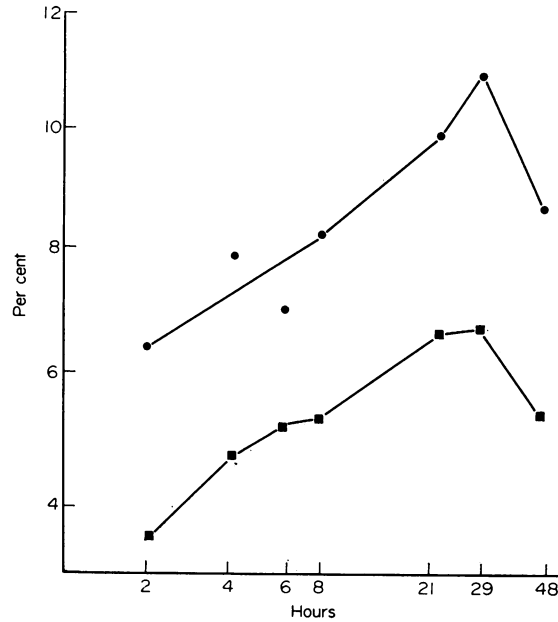

FIG. 1. Arrival of cells at immunized lymph nodes at varying times after cell injection. Mice immunized with picryl chloride (●) and unimmunized (■) mice were injected with ^{51}Cr-labelled cells 24 hours later and the mice killed at the stated time afterwards. The figures show the mean net arrival of ^{51}Cr-labelled cells expressed as a percentage of the radioactivity injected. Each point is based on three mice.

lymph nodes measured. This radioactivity expressed as a percentage of the injected radioactivity gives a measure of the net cell arrival providing there is no elution of ^{51}Cr and all cells are equally labelled.

Fig. 1 shows that the arrival of LNC at unimmunized lymph nodes increased from 3·1 per cent at 2 hours after cell injection to reach a plateau of 6·5 per cent at between 21 and 29 hours. The arrival at immunized lymph nodes was 3–4 per cent units higher at all times. For convenience mice were killed 18–24 hours after cell injection in all subsequent experiments.

KINETICS OF ARRIVAL OF LABELLED NORMAL LYMPH NODE CELLS IN NORMAL AND UNRESPONSIVE MICE

Mice were rendered unresponsive by five injections of picryl sulphonic acid (PSA) and were left for about 6 weeks. Control mice were left untreated. Both groups of mice were sensitized by skin painting with PIC-Cl at various times before the injection of ^{51}Cr-labelled normal LNC. Fig. 2 shows that the arrival of LNC at unimmunized lymph nodes was 5·7 per cent. In contrast the arrival 24 hours after immunization was much greater (13·4 per cent). The arrival of LNC injected 2 days after sensitization was less and the arrival 10 days after immunization was very similar to the arrival at unimmunized lymph nodes. However, immunization of unresponsive mice had less effect on the arrival of normal LNC. One day after immunization the arrival was only 8·4 per cent as compared with 13·4 per cent in the control mice and the arrival at later times after immunization was also depressed as compared to control mice. It was concluded that induction of

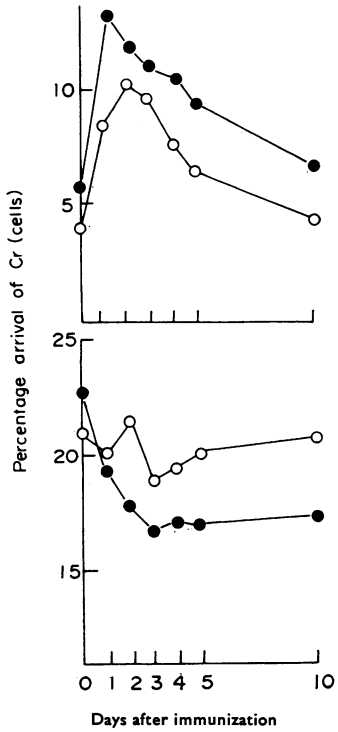

FIG. 2. Kinetics of arrival of labelled normal lymph node cells at draining lymph nodes (upper figure) and spleen (lower figure) in (●) normal and (○) unresponsive mice. Recipients were injected with ^{51}Cr-labelled normal lymph node cells the stated time after immunization with picryl chloride. Each point shows the mean net arrival of ^{51}Cr expressed as a percentage of the radioactivity injected and is based on five recipients.

TABLE 2

DEPRESSION OF ARRIVAL OF NORMAL LYMPH NODE CELLS AT LYMPH NODES IMMUNIZED WITH PICRYL CHLORIDE BY PRETREATMENT WITH A SINGLE INJECTION OF PICRYL SULPHONIC ACID

Treatment of recipients	Sensitizing antigen day 0	Percentage arrival of ^{51}Cr (cells) at draining lymph nodes	
		Experiment 3	Experiment 4
Nil (positive control)	PIC-Cl	10·3 ± 1·27	8·9 ± 0·59
Pretreated with PSA	PIC-Cl	*6·5 ± 0·66	*5·6 ± 0·73
Nil	OX	9·9 ± 1·86	7·9 ± 0·26
Pretreated with PSA	OX	10·6 ± 0·83	8·9 ± 0·77
Nil	Nil	4·5 ± 0·92	3·2 ± 0·26

* Statistically significantly different ($P < 0·001$) from positive control.

The figures show the mean net arrival of ^{51}Cr ± S.D. expressed as a percentage of the radioactivity injected. Each figure is based on four to six recipients. Recipients were pretreated with PSA at −2 days in experiment 3 and −6 days in experiment 4.

unresponsiveness depressed part of the arrival of lymphocytes at lymph nodes caused by skin painting with PIC-Cl.

Fig. 2 also shows that the arrival of injected LNC at the spleen was reduced by immunization with PIC-Cl for at least 10 days. This reduction did not occur in mice rendered

unresponsive by five injections of PSA and at all times after immunization the arrival of LNC at the spleens of unresponsive mice was greater than in normal mice.

Table 3 shows that skin painting with PIC-Cl or the intravenous injection of PSA reduced the arrival of LNC at the spleen. A similar effect is shown in experiment 6 (Table 4). These three experiments taken together suggest that both PSA and PIC-Cl reduce the arrival of labelled LNC at the spleen.

TRANSIENT INCREASE OF ARRIVAL OF LYMPH NODE CELLS AT LYMPH NODES SHORTLY AFTER THE INJECTION OF PICRYL SULPHONIC ACID

PSA like PIC-Cl is chemically reactive and when injected intravenously increases the arrival of normal lymphocytes at lymph nodes. Experiment 1 (Table 1), shows that the arrival of normal LNC at inguinal and brachial lymph nodes is 6·9 per cent and this increases to 9·1 per cent 24 hours after the injection of PSA. This effect is transient and 3 days after the injection of PSA the arrival of LNC has returned to normal. Table 1 shows that this increased arrival of lymphocytes is almost abolished in mice rendered unresponsive by repeated injections of PSA. This suggests that the increased cell arrival is due to an immunological reaction to PSA or picrylated protein and not merely to a chemically induced inflammation. These results were confirmed in experiment 2 (Table 1).

This experiment also shows the movement of cells labelled with ^{125}IUDR taken from lymph nodes immunized with oxazolone (presumptive blast cells). The arrival of these cells was much smaller than the arrival of chromium-labelled cells but increased by a factor of 4 when PSA was given to the recipients 6 hours before cell injection. This increase is entirely absent in animals previously rendered unresponsive to PSA. It was concluded that PSA, like PIC-Cl, increased the arrival of lymph node cells at lymph nodes and that this effect was diminished or abolished in animals rendered unresponsive to PSA.

EFFECT OF SINGLE INJECTION OF PICRYL SULPHONIC ACID ON LYMPH NODE CELL ARRIVAL AT LYMPH NODES IMMUNIZED WITH PICRYL CHLORIDE AND OXAZOLONE

Table 2 confirms that PIC-Cl increased the arrival of LNC at lymph nodes. It also shows that pretreatment with PSA 2 or 6 days beforehand reduced this arrival significantly.

TABLE 3

ARRIVAL OF NORMAL LYMPH NODE CELLS AT LYMPH NODES AND SPLEEN AND THE EFFECT OF PICRYL CHLORIDE AND PICRYL SULPHONIC ACID

Treatment of recipients	Sensitizing antigen day 0	Percentage arrival of ^{51}Cr (cells) at	
		Draining lymph nodes	Spleen
Experiment 5			
Nil	Nil (untreated control)	4·1 ± 0·52	16·2 ± 2·41
−6 hours PSA	Nil	†7·3 ± 0·59	12·9 ± 1·45
−1 day PSA	Nil	†6·7 ± 0·31	12·5 ± 1·69
Nil	PIC-Cl (positive control)	10·1 ± 1·69	14·5 ± 2·28
−6 hours PSA	PIC-Cl	*7·7 ± 1·23	12·6 ± 1·42
−1 day PSA	PIC-Cl	8·5 ± 0·49	12·2 ± 1·33
−2 days PSA	PIC-Cl	9·4 ± 1·2	12·2 ± 1·37
−5 days PSA	PIC-Cl	*8·2 ± 6·75	14·3 ± 0·39

* $P < 0·05$. † $P < 0·001$ statistically significantly different from corresponding control.

The figures show the mean net arrival ± S.D. of ^{51}Cr-labelled normal lymph node cells expressed as a percentage of the radioactivity injected. Each figure is based on four recipients.

TABLE 4

ARRIVAL OF NORMAL LYMPH NODE CELLS AND BLAST CELLS AT LYMPH NODES, SPLEEN AND EARS AND THE EFFECT OF PRETREATMENT WITH PICRYL SULPHONIC ACID ON THE ACTION OF PICRYL CHLORIDE AND 'OXAZOLONE'

Treatment of recipients	Sensitizing antigen Day 0	Antigen used to paint ears Day +1	Percentage arrival of ^{51}Cr (cells) at			Percentage arrival of ^{125}I (cells) at		
			Draining lymph nodes	Spleen	Ears	Draining lymph nodes	Spleen	Ears
Experiment 6								
Nil (control)	PIC-Cl	PIC-Cl	8·6±0·97	17·7±0·62	0·20±0·04	1·57±0·37	2·7±0·54	0·81±0·39
PSA	PIC-Cl	PIC-Cl	*6·9±0·72	17·6±2·26	0·16±0·07	*0·72±0·21	2·1±0·48	1·0 ±0·31
PSA	Nil	PIC-Cl	*5·1±0·39	16·5±1·79	0·28±0·06	*0·46±0·26	1·9±0·16	0·75±0·24
Nil	Nil	PIC-Cl	*5·1±0·79	20·1±1·36	0·22±0·07	*0·52±0·22	2·1±0·06	0·9±0·27
Experiment 7								
Nil (control)	PIC-Cl	PIC-Cl	9·3±1·04	—	0·14±0·02			
PSA	PIC-Cl	PIC-Cl	*7·6±0·43	—	0·16±0·03			
Nil	Nil	PIC-Cl	*4·2±0·44	—	0·15±0·03			
Nil	OX	OX	10·1±0·95	—	0·14±0·02			
PSA	Nil	OX	10·9±0·79	—	0·14±0·02			
Nil	Nil	OX	*4·13±0·66	—	0·16±0·06			

* Statistically significantly different ($P<0·01$) from nil (control) group.

The figures show the mean net arrival of ^{51}Cr-labelled normal lymph node cells, or ^{125}IUDR-labelled lymph node cells taken 3 days after immunization with oxazolone, expressed as a percentage of the radioactivity injected, ±S.D. Each figure is based on five recipients.

Recipients were pretreated with PSA 3 days before immunization in experiment 6 and 6 days before immunization in experiment 7.

This effect was immunological and not due to a general 'anti-inflammatory effect' of PSA as pretreatment with PSA had no effect on the arrival of LNC at lymph nodes immunized with oxazolone.

Experiment 5 (Table 3) shows that PSA decreases arrival of cells following immunization with PIC-Cl when PSA is injected 6 hours beforehand. Under these circumstances PSA alone increases the arrival of LNC at lymph nodes from 4·1 per cent to 7·3 per cent. PIC-Cl alone increases arrival to 10·1 per cent and this is reduced by pretreatment to 7·7 per cent.

ARRIVAL OF NORMAL AND IMMUNIZED LYMPH NODE CELLS AT 'INFLAMMED' EARS

The previous sections showed that there is an immunological and a non-immunological component to the movement of LNC to lymph nodes immunized with PIC-Cl. The immunological component was diminished in animals pretreated with PSA while the non-immunological component was unaffected. Table 4 shows that in two separate experiments the arrival of normal LNC at ears painted with picryl chloride was unaffected by pretreatment with PSA although arrival at lymph nodes was reduced. Experiment 6 (Table 4) also shows similar results using presumptive blast cells (3-day OX-LNC) labelled with ^{125}IUDR. It was concluded that the arrival of LNC at ears inflammed by PIC-Cl in unimmunized mice did not have an immunological component.

DISCUSSION

These results show that there is an increased net arrival of normal lymph node cells and blast cells at lymph nodes immunized with the contact sensitizing agents, picryl chloride and 'oxazolone', as compared with unimmunized lymph nodes. This increased net arrival

is seen both with chromium-labelled normal lymph node cells and cells from nodes immunized with oxazolone which have incorporated the nucleotide IUDR *in vitro*. There are two possible reasons for this increased arrival. First, contact sensitizing agents such as PIC-Cl are chemically reactive and combine with amino and sulphydryl groups and may give rise to chemically induced inflammation. Second, these agents are immunogens and may produce an immunological inflammation. The expectation is that chemical inflammation would be unaffected by the induction of specific unresponsiveness or tolerance while immunological inflammation would be abolished. The results show that the induction of unresponsiveness to PIC-Cl depresses but does not abolish the increased cell arrival caused by painting with PIC-Cl. In contrast, it has no effect on the increased arrival caused by painting with the unrelated antigen oxazolone. This suggests that the increased arrival caused by PIC-Cl has two components—a nonspecific inflammatory component and an immunologically specific component. These two components apparently have the same order of magnitude.

It is interesting that PIC-Cl causes an immunological inflammation in apparently unimmunized mice which is maximal within 44 hours of skin painting. It is, of course, possible that mice have been previously exposed to related antigens such as naturally occurring quinones and Brandriss (1969) found antibody to the picryl group in normal humans. The early occurrence of the immunological component of increased cell arrival suggests that the antibody, or antigen-sensitive cells responsible are preformed and do not come into existence as a result of painting with PIC-Cl. Kelly, Wolstencroft, Dumonde and Balfour (1972) drew attention to the possibility that contact sensitizing agents may interact with lymphocytes in hitherto unimmunized animals and produce factors which cause paracortical plugging in lymph nodes and the accummulation of lymphocytes.

Their findings raise the question whether the increased cell arrival at lymph nodes is due to the liberation of the same lymphokines that occur in the classical delayed hypersensitivity reaction. This question cannot be answered at present but our findings show that there is an increased arrival of lymphoid cells at skin painted with PIC-Cl. However, unlike the increased arrival at lymph nodes, this cannot be abolished by the induction of immunological unresponsiveness to PIC-Cl. Hence the increased arrival at painted skin, unlike the increased arrival at lymph nodes, lacks an immunological component and is entirely due to chemical inflammation. This is true for the increased arrival both of normal lymph node cells and of blast cells produced by immunization with oxazolone—a population known to be rich in inflammatory lymphocytes (Asherson and Allwood, 1972).

There are two ways in which lymphocytes may enter the lymph nodes following painting with picryl chloride. One route is from capillaries in the skin and hence into the afferent lymphatics; the other route is through the capillaries in the lymph node and in particular through the post-capillary venules. Kelly, Wolstencroft, Dumonde and Balfour (1972) have provided some direct evidence that the lymph node capillary route is the most important, by their finding that immunization with the contact sensitizing agent dinitrofluorobenzene does not increase the lymphocyte count in the afferent lymph. The present finding that there is an immunological component in the arrival of lymphocytes at lymph nodes but not in the arrival at painted ears also suggests that most of the lymphocytes arrive at the ear through the lymph node capillaries and not via the afferent lymphatics.

The arrival of injected lymphoid cells at the spleen is reduced by immunization. A simple explanation is that the immunized lymph nodes and the spleen compete for

injected cells. However, this point cannot be established without detailed kinetic studies as it is known that there is an early movement of injected cells to the spleen followed by their movement to lymph nodes (Taub and Lance, 1971).

There is some specific arrival of lymphoid cells at immunized lymph nodes (Emeson and Thursh, 1971; Thursh and Emeson, 1972). However, most of the arrival in nodes immunized with picryl chloride cannot be due to cells potentially reactive to that antigen. The evidence for this is that the immunological component of the arrival is 2–4 per cent and it is difficult to believe that 2–4 per cent of normal lymph node cells or of blast cells produced by immunization with oxazolone are able to react with the picryl group.

It is not surprising that the contact sensitizing agent picryl chloride causes increased net arrival of lymph node cells at lymph nodes. It is more surprising that the intravenous injection of picryl sulphonic acid should have the same effect. There is an immunological component in both cases as the increased cell arrival is less marked in mice rendered unresponsive to the picryl group by the repeated injection of picryl sulphonic acid. The ability of picryl sulphonic acid to cause increased arrival of cells at lymph nodes after intravenous injection may be relevant to clinical situations in which drug ingestion leads to early lymph node enlargement and pain. It also shows that increased net arrival of cells for immunological reasons is not necessarily followed by contact sensitivity.

The ability of contact sensitizing agents to cause a large increase in the cells in the draining lymph node is reminiscent of the effect of Freund's complete adjuvant and other adjuvants (Dresser, Taub and Krantz, 1970). The arrival caused by picryl chloride in Freund's adjuvant, however, rises between 2 and 11 days after immunization (Allwood and Asherson, 1972). In contrast, the arrival caused by painting with picryl chloride is maximal at 1 day after immunization. It would be interesting to know whether agents which attract cells to lymph nodes have an adjuvant action and whether the number of cells attracted early in an immune response is an important determinant of its magnitude (Taub and Gershon, 1972).

ACKNOWLEDGMENTS

R.M.R.B. was a junior research fellow of the Medical Research Council.

REFERENCES

ALLWOOD, G. G. and ASHERSON, G. L. (1972). 'Depression of delayed hypersensitivity by pretreatment with Freund-type adjuvants. III. Depressed arrival of lymphoid cells at recently immunized lymph nodes in mice pretreated with adjuvants.' *Clin. exp. Immunol.*, **11**, 579.

ASHERSON, G. L. and ALLWOOD, G. G. (1972). 'Inflammatory lymphoid cells. Cells in immunized lymph nodes that move to sites of inflammation.' *Immunology*, **22**, 493.

ASHERSON, G. L., ZEMBALA, M. and BARNES, R. M. R. (1971). 'The mechanism of immunological unresponsiveness to picryl chloride and the possible role of antibody mediated depression.' *Clin. exp. Immunol.*, **9**, 111.

BRANDRISS, M. W. (1969). 'Antibody-like activity to the 2,4-dinitrophenyl group in normal human sera.' *Nature (Lond.)*, **221**, 960.

DRESSER, D. W., TAUB, R. N. and KRANTZ, A. R. (1970). 'The effects of localized injection of adjuvant material on the draining lymph node. II. Circulating lymphocytes.' *Immunology*, **18**, 663.

EMESON, E. E. and THURSH, D. R. (1971). 'Immunologically specific retention of long-lived lymphoid cells in antigenically stimulated lymph nodes.' *J. Immunol.*, **106**, 635.

KELLY, R. H., WOLSTENCROFT, R. A., DUMONDE, D. C. and BALFOUR, B. M. (1972). 'Role of lymphocyte activation products (LAP) in cell mediated immunity. II. Effects of lymphocyte activation products on lymph node architecture and evidence for peripheral release of LAP following antigenic stimulation.' *Clin. exp. Immunol.*, **10**, 49.

TAUB, R. N. and GERSHON, R. K. (1972). 'The effect of

ocalized injection of adjuvant material on the draining lymph node. III. Thymus dependence.' *J. Immunol.*, **108**, 377.

TAUB, R. N. and LANCE, E. M. (1971). 'Effects of lymphoid depletion on the distribution of Cr-labelled lymph node cells in mice.' *Transplantation*, **11**, 536.

THURSH, D. R. and EMESON, E. E. (1972). 'The immunologically specific retention of recirculating long-lived lymphocytes in lymph nodes stimulated by xenogeneic erythorocytes.' *J. exp. Med.*, **135**, 754.

ZATZ, M. M. and LANCE, E. M. (1971a). 'The distribution of ^{51}Cr-labelled lymphocytes into antigen-stimulated mice.' *J. exp. Med.*, **134**, 224.

ZATZ, M. M. & LANCE, E. M. (1971b). 'Lymphocyte trapping in tolerant mice.' *Nature: New Biology*, **234**, 253.